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CONTENTS

Continued on back cover

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Mulberry leaf age and varietal influence on the haemolymph proteins of silkworm, *Bombyx mori* L.

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ABSTRACT: Tested the effect of leaves of different age group of mulberry varieties on haemolymph proteins of silkworm, *Bombyx mori*. Larvae of both sexes fed on tender leaves of varieties ichinose, kosen, MR2, S34, M5 and C1 showed maximum accumulation of all the polypeptides when compared to larvae fed on medium and coarse leaves of these varieties. Among varieties, larvae fed on leaves of ichinose showed maximum accumulation of polypeptides than that in the other varieties. Electro-blotted gels probed with antibodies for storage proteins also confirmed the results. Hence investigations reveal the nutritional influence of host plants on the development of silkworm.

KEYWORDS: Mulberry varieties, haemolymph proteins, storage proteins, nutritional status, *Bombyx mori*

INTRODUCTION

In insects, haemolymph proteins have a diversity of functions like metabolic, endocrine, reproductive and immunological. Haemolymph serves as the transport milieu for the exchange of essential materials between cells, tissues and organs (Mullins, 1985; Karpells *et al.*, 1990; Lovallo *et al.*, 2000). During insect metamorphosis, profound biochemical changes occur in the haemolymph, in particular, the concentrations of certain pools of proteins and amino acids undergo changes. Most importantly, special classes of haemolymph proteins known as storage proteins undergo significant changes (Mullins, 1985; Levenbook, 1985; Nagata and Kobayashi, 1990). It has been reported that these larval storage proteins accumulate in the organism at the time of abundant dietary intake and then acts as protein reserves during non-feeding phase of development. These findings stress the importance of larval haemolymph storage

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proteins in growth and development of insects (Roberts and Brock, 1981; Levenbook, 1985; Nagata and Kobayashi, 1990). According to Nagata and Kobayashi (1990) these storage proteins function as the main reservoirs for the supply of amino acids, both at the time of larval moults and during metamorphosis.

Early literature on the protein components of the haemolymph in *B. mori* mainly explained their synthesis and influence of hormones. However, ever-since the demonstration of their significant role in the growth and development of insects, several studies have been performed to determine the influence of nutrition on the development of *B. mori* (Mullins, 1985; Watanabe and Horie, 1980; Riddiford and Hice, 1985; Nagata and Kobayashi, 1990; Krishnan *et al.*, 1995). Studies of several workers (Chawla, 1969; Krishnaswami, 1979; Sinha *et al.*, 1986; Sikdar, 1993, Krishnan *et al.*, 1995) have shown that the quality of nutrient is altered depending on the age of the leaves as well as on the variety of mulberry. In the present work the effect of feeding different variety mulberry leaves of various age groups on the haemolymph proteins, especially, the storage proteins in the silkworm *B. mori* was assessed.

MATERIALS AND METHODS

Representative leaf samples [tender–1st to 7th leaf, medium–8th to 15th leaf and coarse/non-senescent–16th to 23rd leaf from the top of the bush (Ito and Arai, 1963) of the selected mulberry varieties [ichinose, kosen (exotic), MR2, S34, M5 and C1 (indigenous)] were collected 45 days after pruning for feeding the silkworms. The crossbreed of silkworm larvae-$LxNB_4D_2$ was used for the present study. The larvae after hatching were brushed and divided into different groups and were reared on leaves of different age groups of selected mulberry varieties. The larvae were fed four times a day with respective types of leaves at a temperature of 25 ± 1°C and RH 70–75%.

Haemolymph samples of 5-day old last larval instar were collected from all the feeding groups. Larvae were bled by wounding the distal region of the prothoracic leg and the haemolymph was drained into an eppendrof tube kept in crushed ice with a sufficient quantity of phenylthiourea to saturate the haemolymph. The haemolymph samples were then diluted with phosphate buffered saline (pH 7.0) with 1mM phenylmethylsulfonyl fluoride (PMSF) in 1 : 5 ratio. The mixture was then centrifuged at 5000 rpm for 5 min to remove the haemocytes and other tissue debris. Supernatant was then subjected to gel electrophoresis (SDS-PAGE) using equal quantities of samples by following the method of (Laemmli, 1970). Standard marker proteins (Dalton mark VII-LTM, Sigma molecular weight markers) were run for comparison.

Gels were run parallely and electrophoretically blotted (Towbin *et al.*, 1979) onto a nitrocellulose membrane. They were then incubated with antibodies for storage proteins (both SP1 and SP2). The antibodies were obtained as a kind gift from Prof. O. Yamashita, Nagoya University, Nagoya, Japan.

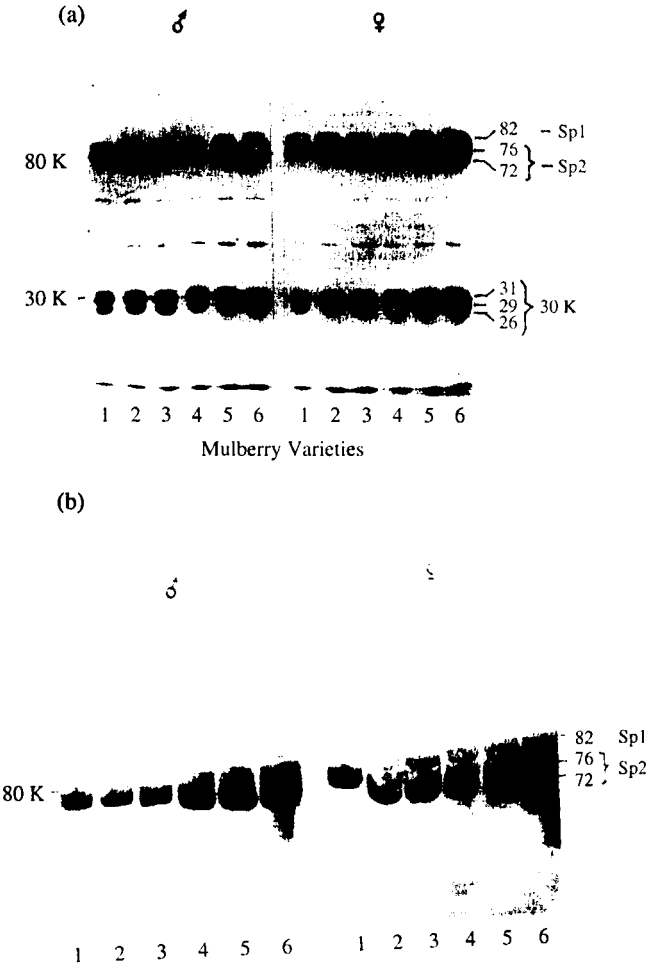

FIGURE 1. SDS-Polyacrylamide gel electrophoretic profile (a) and immunoblot (b) of haemolymph proteins in 7th day fifth instar larvae of silkworm, *Bombyx mori*, fed on tender leaves of different varieties of mulberry. Lane 1-C1, lane 2-M5, lane 3-S34, lane 4-MR2, lane 5-kosen and lane 6-ichinose.

RESULTS

The protein profile of the haemolymph (Figs 1a, 2a and 3a) showed several polypeptides with molecular weights ranging from 200-18 kDa and was found in the haemolymph of all the feeding groups. In all the samples, seven polypeptides constituted the bulk of the coomassie brilliant blue stained gel and had apparent

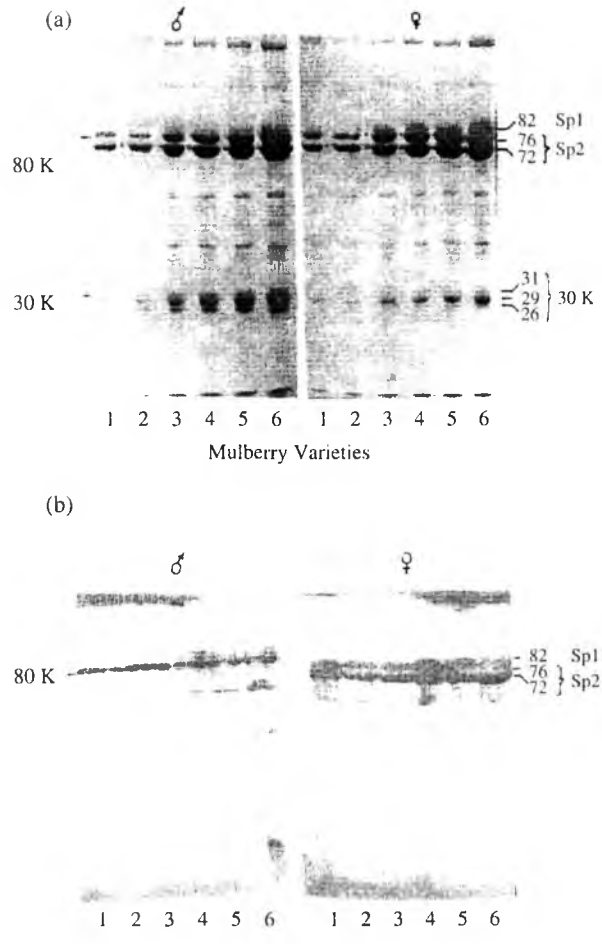

FIGURE 2. SDS-Polyacrylamide gel electrophoretic profile (a) and immunoblot (b) of haemolymph proteins in 7th day fifth instar larvae of silkworm, *Bombyx mori*, fed on medium leaves of different varieties of mulberry. Lane 1-C1, lane 2-M5, lane 3-S34, lane 4-MR2, lane 5-kosen and lane 6-ichinose.

molecular weights of 200, 82, 76, 72, 31, 29 and 26 kDas. A polypeptide localized around 200 kDa region was identified as the heavy subunit of lipoprotein. The two polypeptides [SP1 (32 kDa) and SP2 (76 and 72 kDas)] that electrophoresed to the 80 kDa region were identified as storage proteins. They were found the most abundant polypeptides in the haemolymph. In the electrophorogram, the storage protein 1 (SP1: 32 kDa female specific polypeptide) was found only in the haemolymph of the female larvae; the storage protein 2 with two subunits (SP2: 76 and 72 kDa) was

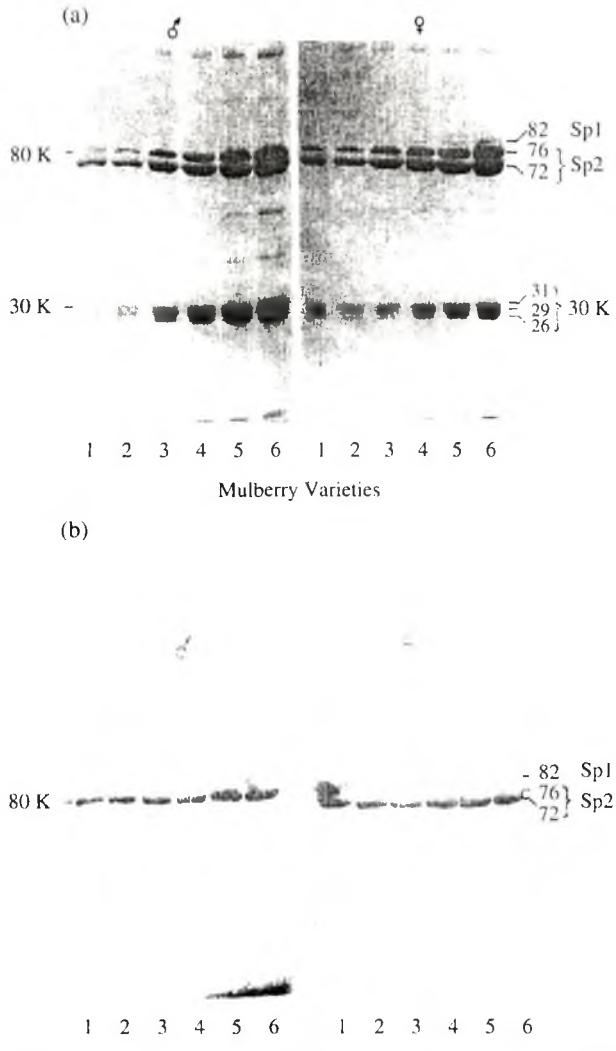

FIGURE 3. SDS-Polyacrylamide gel electrophoretic profile (a) and immunoblot (b) of haemolymph proteins in 7th day fifth instar larvae of silkworm, *Bombyx mori*, fed on coarse leaves of different varieties of mulberry. Lane 1-C1, lane 2-M5, lane 3-S34, lane 4-MR2. lane 5-kosen and lane 6-ichinose.

found in both the sexes. The fourth group of polypeptides, which localized at the 30 kDa region consisted of three subunits namely 31, 29 and 26 kDa polypeptides. This 30 kDa polypeptide was found second most abundant haemolymph protein next to the storage proteins. During development of fifth instar larva (from day 1 to day 7)

intensity of haemolymph proteins particularly that of the storage proteins and 30 kDa protein steadily increased reaching a maximum level before the onset of spinning i.e. on the 7th day.

The larvae of both sexes that were fed on tender leaves of ichinose, kosen, MR2, S34, M5 and C1 showed the greatest staining intensities of polypeptide bands (Fig. 1a) when compared to that in the larvae fed on medium and coarse leaves of the same varieties (Figs 2a and 3a). Among varieties, larvae that were fed on tender, medium and coarse leaves of ichinose showed the most intensely stained polypeptides than did the larvae fed on leaves of other varieties. The parallelly electroblotted gels (samples collected on the 7th day of fifth instar) probed with antibodies developed against the storage proteins (SP1 and SP2) also showed strongly reacting antigens of SP1 and SP2 in the haemolymph of larvae fed on the tender leaves of all the varieties and on the different leaves of ichinose. Weakly reactive bands of storage proteins were also observed in larvae of both sexes that were fed on medium and coarse leaves of other mulberry varieties (Figs 1b, 2b and 3b).

DISCUSSION

Storage proteins and 30 kDa polypeptides were found in significantly greater concentrations in the haemolymph of the larvae fed on tender leaves of all the varieties, specially those of ichinose. Riddiford and Hice (1985); Nagata and Kobayashi (1990); Krishnan *et al.* (1995) all reported that accumulation of storage proteins is greatly influenced by the nutritional richness of the diet. Further, they proved the quantum of storage proteins in the larvae fed on the low protein diet was far less than the larvae fed on high protein diet. Biochemical analysis of the mulberry leaves revealed that protein was the predominant nutrient available in the tender leaves (Janarthanan *et al.*, 2000). This is the essential point to note that the leaf protein plays a major role in insect growth and development (Mattson, 1980; Scriber and Slansky, 1981). The observation made in the present investigation strongly suggests that, the levels of haemolymph proteins especially the storage proteins that are responsible for various physiological and life processes in the larvae of silkworms are inexorably linked to the quality of nutrient. The empirical data also suggested the nutritional and physiological requirements of the silkworm could be fulfilled by providing mixed varietal feeding to silkworms (Nagaraju, 2002).

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Development and reproductive potential of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) on various plant parts of cotton cultivars/hybrid

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ABSTRACT: The development and reproductive potential of *Helicoverpa armigera* (Hubner) on different plant parts namely foliage, squares and bolls of different cotton varieties *viz.*, LRA 5166, SVPR 2, KC 2 and K 11 and a hybrid RCH 2 were studied. The variety K 11 recorded longer larval, pupal, pre-oviposition, incubation, and developmental periods, shorter oviposition and post-oviposition periods and low larval and pupal weight, fecundity, adult longevity, per cent eclosion, pupation and adult emergence compared to other varieties/hybrid. The significant variations in larval period and weight, pupal period and weight, pupation, adult emergence, pre-oviposition, fecundity, longevity, incubation period and developmental period of *H. armigera* on various parts of the cultivars/hybrid suggest differences in the nutritional and biochemical constituents. The larvae fed on bolls showed good growth, development and reproductive potential followed by the larvae fed on squares and foliage.

KEYWORDS: Growth and development, cotton cultivars, *Helicoverpa armigera*

INTRODUCTION

The American bollworm, *Helicoverpa armigera* (Hubner) is a serious pest of more than 200 species of plants (Zalucki *et al.*, 1994), including economically important crops like cotton, tomato, sorghum, groundnut, chickpea, pigeonpea, sunflower, maize, etc. Among the insect pests of cotton, *H. armigera* is considered to be one of the most important pests that cause severe damage to cotton crop and substantial loss in seed cotton yield. The estimates of annual economic loss due to this pest include

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US $ 1500 million for cotton, worldwide (International Cotton Advisory Committee, 1998). A number of cultivars/varieties/hybrids are available in cotton. The information on the growth and development of *H. armigera* on different cultivars / hybrids is not adequate. Hence the present investigation was undertaken to elucidate the influence of various plant parts (foliage, squares and bolls) of cotton cultivars belonging to *arboreum* (K 11) and *hirsutum* (LRA 5166, SVPR 2 and KC 2) groups and a hybrid (RCH 2) on the development and reproductive potential of *H. armigera*.

MATERIALS AND METHODS

Development and reproductive potential of *H. armigera* on various plant parts of different cultivars/hybrid

The development of *H. armigera* on foliage, squares and bolls of four cotton cultivars *viz.*, LRA 5166, SVPR 2, K 11 and KC 2 and a hybrid RCH 2 was investigated. Neonate larvae obtained from the stock culture maintained in insectary were used for studying the various larval parameters. The larvae were reared singly on leaves, squares and bolls (obtained from upper canopy of pesticide untreated cotton plants) of four cotton cultivars and a hybrid, until pupation. The larvae were transferred every day to sterilized rearing containers (7 × 7 cm), provisioned with freshly excised, unsprayed plant parts from the particular varieties/hybrid. For each treatment, three replications were maintained by having twenty larvae per replication. Observations on mean larval period, larval weight, pupal period and pupal weight were recorded. Fully grown larvae were weighed when they ceased feeding and moved their heads sideways to locate pupation sites. Pupal weight was recorded, five days after pupation in order to avoid injury to pupae. Male and female pupae were separated (after sexing), and kept in separate containers. Adults ecloding from pupae were kept singly in round perspex containers (7 × 7 cm) and were provided with 10 per cent honey solution in plastic stopper to facilitate feeding. The male and female moths were paired (1 : 1) on third night after emergence in individual mating jar, which also served as oviposition jar (11.5 × 9 cm). A strip of tissue paper was placed on the sides of the jar in such a way that it hanged along the internal wall of the jar. The tissue paper lining at the bottom and side walls of jar provided surface for perching as well as egg laying. The mouth of the jar was covered with muslin cloth, which was held tightly with a rubber band. This prevented escape of the moths from jars and allowed adequate ventilation.

Pre-oviposition, oviposition and post-oviposition periods, developmental period, fecundity and longevity of adult moths were recorded. Incubation and eclosion were observed for 150 eggs (50 eggs for each of three replicates) obtained randomly from the moths of respective treatments. The growth indices, *viz.*, growth index (Srivastava, 1959; Pandey *et al.*, 1968), Howe's growth index (Howe, 1979) and suitability index (Murali Baskaran *et al.*, 1994) were worked out for the larvae reared on bolls only. The experiment was carried out at 28 ± 2 °C and 65 ± 5 per cent RH at the insectary, Department of Entomology during November 2001.

TABLE 1. Larval period and weight of *H. armigera* reared on foliage, squares and bolls of different cotton varieties/hybrid

Biological parameters*	Plant part	Varieties/hybrid					
		LRA 5166	SVPR 2	KC 2	K 11	RCH 2 (Hybrid)	Mean
Larval period (days)	Foliage	21.20 $(4.60)^{aBC}$	21.50 $(4.64)^{aBC}$	22.70 $(4.76)^{aAB}$	23.20 $(4.82)^{aA}$	21.00 $(4.60)^{aC}$	21.92 $(4.68)^{a}$
	Squares	19.40 $(4.40)^{bB}$	19.70 $(4.44)^{bB}$	20.30 $(4.51)^{bAB}$	21.60 $(4.65)^{bA}$	19.60 $(4.40)^{bB}$	20.12 $(4.48)^{b}$
	Bolls	16.20 $(4.02)^{cB}$	16.40 $(4.05)^{cB}$	17.20 $(4.15)^{cAB}$	18.40 $(4.29)^{cA}$	16.00 $(4.02)^{cB}$	16.84 $(4.10)^{c}$
	Mean	18.93 $(4.34)^{B}$	20.07 $(4.38)^{B}$	20.08 $(4.47)^{AB}$	21.07 $(4.58)^{A}$	18.87 $(4.34)^{B}$	
Larval weight (mg)	Foliage	568.70 $(2.75)^{bA}$	564.20 $(2.75)^{bA}$	546.60 $(2.74)^{bA}$	482.40 $(2.68)^{bB}$	570.80 $(2.76)^{bA}$	546.57 $(2.74)^{b}$
	Squares	602.30 $(2.78)^{aA}$	599.80 $(2.78)^{aA}$	580.40 $(2.76)^{abA}$	505.20 $(2.70)^{abB}$	606.40 $(2.78)^{abA}$	578.83 $(2.76)^{ab}$
	Bolls	625.40 $(2.79)^{aA}$	610.20 $(2.79)^{aA}$	595.50 $(2.77)^{aA}$	527.60 $(2.72)^{aB}$	630.10 $(2.80)^{aA}$	597.76 $(2.78)^{a}$
	Mean	598.80 $(2.78)^{A}$	591.44 $(2.77)^{A}$	574.20 $(2.76)^{A}$	505.07 $(2.70)^{B}$	602.43 $(2.78)^{A}$	

*Mean of three replications based on 20 observations; Figures in parentheses of first parameter are square root transformed values and second parameter are log transformed values. Means in a column/row followed by same small/capital letter(s) respectively are not significantly different ($P = 0.05$) by DMRT.

RESULTS

Larval period and weight

The mean larval period significantly varied for the larvae reared on different plant parts of cotton, foliage (21.92 days), squares (20.12 days) and bolls (16.84 days). There were significant differences observed for larval period among the varieties/hybrid. Maximum larval period was noticed for larvae reared on K 11 cotton variety The larvae reared on LRA 5166, RCH 2 and SVPR 2 were on par with reference to larval period.

Weight for larvae reared on bolls (597.76 mg) significantly differed from that of larvae reared on squares (578.83 mg) and foliage (546.57 mg). Weight of K 11 reared larvae (505.07 mg) significantly differed from others. The larvae reared on other varieties/hybrid were on par with each other (Table 1).

Pupal period and weight

The pupal period for larvae reared on different plant parts varied significantly. The larvae reared on bolls, squares and foliage took 13.88, 14.26 and 15.50 days to

TABLE 2. Pupal period and weight of *H. armigera* reared on foliage, squares and bolls of different cotton varieties/hybrid

Biological parameters*	Plant part	Varieties/hybrid					
		LRA 5166	SVPR 2	KC 2	K 11	RCH 2 (Hybrid)	Mean
Pupal period (days)	Foliage	15.10 (3.89)^{aB}	15.20 (3.90)^{aAB}	15.80 (3.91)^{aAB}	16.30 (4.04)^{aA}	15.00 (3.87)^{aB}	15.50 (3.93)^{a}
	Squares	13.50 (3.67)^{bB}	13.90 (3.73)^{bBC}	14.80 (3.85)^{bAB}	15.30 (3.91)^{bA}	13.80 (3.71)^{bBC}	14.26 (3.78)^{b}
	Bolls	13.20 (3.63)^{bB}	13.50 (3.67)^{bB}	14.20 (3.77)^{bAB}	14.90 (3.86)^{aA}	13.60 (3.69)^{bB}	13.88 (3.72)^{b}
	Mean	13.93 (3.73)^{B}	14.20 (3.77)^{B}	14.93 (3.86)^{AB}	15.50 (3.94)^{A}	14.13 (3.76)^{B}	
Pupal weight (mg)	Foliage	232.60 (2.37)^{bA}	231.80 (2.36)^{bA}	224.60 (2.35)^{bA}	206.70 (2.31)^{bB}	235.90 (2.37)^{bA}	226.32 (2.35)^{b}
	Squares	280.20 (2.45)^{aA}	278.4 (2.44)^{aA}	258.90 (2.41)^{aB}	239.50 (2.38)^{aC}	284.60 (2.45)^{aA}	268.32 (2.43)^{a}
	Bolls	290.80 (2.46)^{aA}	291.60 (2.46)^{aA}	269.70 (2.43)^{aB}	258.40 (2.41)^{aB}	297.30 (2.47)^{aA}	281.56 (2.45)^{a}
	Mean	267.87 (2.43)^{A}	267.27 (2.42)^{A}	251.07 (2.40)^{AB}	234.87 (2.37)^{B}	272.60 (2.43)^{A}	

*Mean of three replications based on 10 observations. Figures in parentheses of first parameter are square root transformed values and second parameter are log transformed values. Means in a column/row followed by same small/capital letter(s) respectively are not significantly different ($P = 0.05$) by DMRT.

complete the pupal stage, respectively. The larvae reared on squares and bolls were on par with each other. The differences in pupal period on different plant parts among the varieties/hybrid varied significantly. LRA 5166, SVPR 2 and RCH 2 reared larvae showed lower pupal periods compared to KC 2 (14.93 days) and K 11 (15.50 days).

Pupal weight reached its maximum when larvae reared on bolls (281.56 mg) and squares (268.32 mg), which were on par with each other. Minimum pupal weight (226.32 mg) was recorded for pupae of larvae reared on foliage. The larvae reared on different varieties/hybrid differed significantly regarding pupal weight. LRA 5166, SVPR 2 and RCH 2 were on par among themselves except K 11 (Table 2).

Pre-oviposition, oviposition and post-oviposition periods

Pre-oviposition periods of the adults obtained from the larvae rared on different plant parts significantly differed when reared on squares (3.00 days), bolls (3.06 days) and squares (3.22 days). The differences in the pre-oviposition periods of adults obtained from larvae reared on different parts of cotton among the varieties/hybrid were significant. LRA 5166 (3.00 days), SVPR 2 (3.00 days) and RCH 2 (2.97 days) relatively short and were on par as compared to KC 2 and K 11.

TABLE 3. Pre-oviposition, oviposition and post-oviposition periods of *H. armigera* reared on foliage, squares and bolls of different cotton varieties/hybrid

Biological parameters*	Plant part	Varieties/hybrid					
		LRA 5166	SVPR 2	KC 2	K 11	RCH 2 (Hybrid)	Mean
Pre-oviposition period (days)	Foliage	3.20 $(1.79)^{aAB}$	3.10 $(1.76)^{aB}$	3.40 $(1.84)^{aA}$	3.40 $(1.84)^{aA}$	3.00 $(1.73)^{aB}$	3.22 $(1.79)^{a}$
	Squares	2.80 $(1.67)^{cC}$	3.00 $(1.73)^{bABC}$	3.10 $(1.76)^{bAB}$	3.20 $(1.79)^{aA}$	2.90 $(1.70)^{aBC}$	3.00 $(1.73)^{b}$
	Bolls	3.00 $(1.73)^{bAB}$	2.90 $(1.70)^{bB}$	3.20 $(1.79)^{abA}$	3.20 $(1.79)^{aA}$	3.00 $(1.73)^{aAB}$	3.06 $(1.75)^{ab}$
	Mean	3.00 $(1.73)^{B}$	3.00 $(1.73)^{B}$	3.23 $(1.80)^{A}$	3.27 $(1.81)^{A}$	2.97 $(1.72)^{B}$	
Oviposition period (days)	Foliage	10.10 $(3.18)^{bA}$	9.80 $(3.13)^{cA}$	8.40 $(2.90)^{cB}$	7.50 $(2.74)^{cC}$	9.90 $(3.15)^{bA}$	9.14 $(3.02)^{c}$
	Squares	10.80 $(3.29)^{aA}$	10.40 $(3.22)^{bA}$	9.20 $(3.03)^{bB}$	8.80 $(2.97)^{bB}$	10.94 $(3.30)^{aA}$	10.02 $(3.16)^{b}$
	Bolls	11.20 $(3.35)^{aA}$	11.40 $(3.38)^{aA}$	10.10 $(3.18)^{aB}$	9.40 $(3.07)^{aB}$	11.40 $(3.38)^{aA}$	10.70 $(3.23)^{a}$
	Mean	10.70 $(3.27)^{A}$	10.53 $(3.24)^{A}$	9.23 $(3.04)^{B}$	8.57 $(2.92)^{B}$	10.73 $(3.27)^{A}$	
Post-oviposition period (days)	Foliage	3.20 $(1.79)^{bA}$	3.30 $(1.82)^{bA}$	2.70 $(1.64)^{bB}$	2.20 $(1.48)^{cC}$	3.20 $(1.79)^{bA}$	2.92 $(1.70)^{c}$
	Squares	3.40 $(1.84)^{bA}$	3.30 $(1.82)^{bA}$	2.80 $(1.67)^{bB}$	2.60 $(1.61)^{bB}$	3.50 $(1.87)^{aA}$	3.12 $(1.76)^{b}$
	Bolls	3.80 $(1.95)^{aA}$	3.70 $(1.92)^{aA}$	3.20 $(1.79)^{aB}$	2.90 $(1.73)^{aB}$	3.60 $(1.90)^{aA}$	3.44 $(1.86)^{a}$
	Mean	3.47 $(1.86)^{A}$	3.43 $(1.85)^{A}$	2.90 $(1.70)^{B}$	2.57 $(1.61)^{C}$	3.43 $(1.85)^{A}$	

*Mean of three replications based on 5 observations. Figures in parentheses are square root transformed values. Means in a column/row followed by same small/capital letter(s) respectively are not significantly different ($P = 0.05$) by DMRT.

Oviposition period for the larvae reared on different plant parts significantly varied and was minimum on foliage (9.14 days), followed by squares (10.02 days) and bolls (10.70 days). LRA 5166, SVPR 2 and RCH 2 were on par and differed significantly from the oviposition periods recorded in the varieties K 11 and KC 2. Minimum post-oviposition period was recorded on foliage (2.92 days), followed by squares (3.12 days) and bolls (3.41 days) and they differed significantly among them. There were significant differences existed among the varieties/hybrid in post-oviposition periods on different plant parts. The post-oviposition period observed for LRA 5166 (3.47 days), SVPR 2 (3.43 days) and RCH 2 (3.43 days) were on par and K 11 and KC 2 differed significantly (Table 3).

Fecundity and adult longevity

The eggs laid by adults emerged from bolls (697.32 eggs/female) and squares (670.48 eggs/female) were on par, whereas the eggs laid by adults emerged from foliage (581.78 eggs/female) differed significantly. The differences in fecundity among the varieties/hybrid for larvae reared on different plant parts showed significance. Fecundity was less for the adults emerged from K 11 (581.93 eggs), which was on par with KC 2 (618.07 eggs). Adult longevity significantly varied when larvae reared on different plant parts of cotton varieties/hybrid. Longevity of male was low when the larvae reared on foliage (12.84 days), squares (13.78 days) and maximum on bolls (15.41 days). The male longevity for larvae reared on LRA 5166(14.63 days), SVPR 2 (14.57 days) and RCH 2 (14.57 days), were on par with each other and significantly differed from that of KC 2 (13.53 days) and K 11 (12.70 days) which were on par. The female longevity was more when larvae were fed on bolls (16.62 days), squares (15.26 days) and less on foliage (14.33 days) and each differed significantly from each other. When the larvae reared on LRA 5166 (16.13 days), SVPR 2 (16.23 days) and RCH 2 (16.27 days) were on par with each other and significantly recorded maximum female adult longevity followed by KC 2 (14.77 days) and K 11 (13.57 days) (Table 4).

Incubation period of eggs and eclosion

Maximum incubation period of eggs was recorded from larvae reared on cotton squares (3.32 days) and minimum on foliage (3.02 days) and they were significantly different. The incubation period was maximum when larvae reared on plant parts of K 11 (3.37 days) and KC 2 (3.33 days) which were on par, whereas the minimum was observed with LRA 5166 (2.97 days), RCH 2 (2.97 days) and SVPR 2 (3.07 days) and which were on par with each other. The egg eclosion ranged from 81.20 (foliage) to 88.53 per cent (bolls) in different plant parts of cotton varieties/hybrid and they significantly differed with each other. The egg eclosion was higher when the larvae reared on RCH 2 (90.67%), SVPR 2 (88.22%), LRA 5166 (88.00%), which were on par with each other and significantly differed with that of KC 2 (82.00%) and K 11 (77.11%) which were on par with each other (Table 5).

Successful per cent pupation, adult emergence and developmental period

Mean successful pupation ranged from 61.33 (foliage) to 72.67 (bolls) per cent when the insect was reared on different plant parts and they were significantly different among them. The mean successful pupation was maximum when larvae reared on RCH 2 (73.33%) followed by SVPR 2 (70.00%) and LRA 5166 (69.44%) and all were on par with each other. KC 2 (63.89%) and K 11 (57.78%) recorded less and they were on par with each other. The adult emergence did not show significant differences when larvae reared on foliage (71.33%), squares (74.00%) and bolls (71.67%). However, differences in adult emergence among the varieties/hybrid existed significantly. Maximum adult emergence was observed with SVPR 2 (78.89%) and minimum was on K 11 (65.56%) (Table 6).

TABLE 4. Fecundity and adult longevity of *H. armigera* for larvae reared on foliage, squares, and bolls of different cotton varieties/hybrid

Biological parameters*	Plant part	Varieties/hybrid					
		LRA 5166	SVPR 2	KC 2	K 11	RCH 2 (Hybrid)	Mean
Fecundity (eggs)	Foliage	614.80 (2.79)^{bA}	596.30 (2.78)^{bA}	543.20 (2.73)^{bB}	526.40 (2.72)^{bB}	628.20 (2.80)^{bA}	581.78 (2.76)^{b}
	Squares	704.30 (2.85)^{aA}	698.20 (2.84)^{aA}	632.80 (2.80)^{aB}	602.60 (2.78)^{aB}	714.50 (2.85)^{aA}	670.48 (2.83)^{a}
	Bolls	730.30 (2.86)^{aAB}	718.60 (2.86)^{aAB}	678.20 (2.83)^{aB}	616.80 (2.79)^{aC}	742.70 (2.87)^{aA}	697.32 (2.84)^{a}
	Mean	683.13 (2.83)^{A}	671.03 (2.83)^{A}	618.07 (2.79)^{B}	581.93 (2.76)^{B}	695.13 (2.84)^{A}	
Adult longevity of male (days)	Foliage	13.80 (3.71)^{bA}	13.40 (3.66)^{cA}	12.00 (3.46)^{cB}	11.50 (3.39)^{cB}	13.50 (3.67)^{cA}	12.84 (3.58)^{c}
	Squares	14.20 (3.77)^{bA}	14.30 (3.78)^{bA}	13.50 (3.67)^{bA}	12.40 (3.52)^{bB}	14.40 (3.79)^{bA}	13.78 (3.71)^{b}
	Bolls	15.90 (3.99)^{aA}	16.00 (4.00)^{aA}	15.10 (3.89)^{aA}	14.20 (3.77)^{aB}	15.80 (3.97)^{aA}	15.41 (3.92)^{a}
	Mean	14.63 (3.82)^{A}	14.57 (3.81)^{A}	13.53 (3.67)^{B}	12.70 (3.56)^{B}	14.57 (3.81)^{A}	
Adult longevity of female (days)	Foliage	15.20 (3.90)^{bA}	15.20 (3.90)^{bA}	13.80 (3.71)^{bB}	11.90 (3.45)^{cC}	15.40 (3.92)^{bA}	14.33 (3.77)^{c}
	Squares	16.00 (4.00)^{abA}	16.10 (4.01)^{bA}	14.40 (3.79)^{bB}	13.60 (3.69)^{bB}	16.20 (4.02)^{bA}	15.26 (3.90)^{b}
	Bolls	17.20 (4.15)^{aAB}	17.40 (4.17)^{aA}	16.10 (4.01)^{aBC}	15.20 (3.90)^{aC}	17.20 (4.15)^{aA}	16.62 (4.08)^{a}
	Mean	16.13 (4.01)^{A}	16.23 (4.03)^{A}	14.77 (3.84)^{B}	13.57 (3.68)^{C}	16.27 (4.03)^{A}	

*Mean of three replications based on 5 observations. Figures in parentheses of first parameter are log transformed values and second and third parameters are square root transformed values. Means in a column/row followed by same small/capital letter(s) respectively are not significantly different ($P = 0.05$) by DMRT.

The mean developmental period significantly differed for larvae reared on plant parts and ranged from 33.80 (bolls) to 40.44 days (foliage). The mean developmental period was minimum on LRA 5166 (35.83 days) and maximum on K 11 (39.93 days) and there were significant differences in developmental period among the varieties/hybrid (Table 6).

Indices for growth and development of *H. armigera* on different varieties/hybrid

Growth index ranged from 3.623 (K 11) to 4.792 (RCH 2) among the varieties/hybrid for *H. armigera*. Howe's, growth index was high in LRA 5166 and RCH 2 (0.058)

TABLE 5. Incubation period and eclosion of eggs of *H. armigera* moths of larvae reared on foliage, squares and bolls of different cotton varieties/hybrid

Biological parameters*	Plant part	Varieties/hybrid					
		LRA 5166	SVPR 2	KC 2	K 11	RCH 2 (Hybrid)	Mean
Incubation period (days)	Foliage	2.80 (1.67)^{bB}	3.00 (1.73)^{bAB}	3.20 (1.79)^{bA}	3.20 (1.79)^{bA}	2.90 (1.70)^{bB}	3.02 (1.73)^{b}
	Squares	3.20 (1.79)^{aB}	3.20 (1.79)^{aB}	3.50 (1.87)^{aA}	3.60 (1.90)^{aA}	3.10 (1.76)^{aB}	3.32 (1.82)^{a}
	Bolls	2.90 (1.70)^{bB}	3.00 (1.73)^{bB}	3.30 (1.82)^{aA}	3.30 (1.82)^{bA}	2.90 (1.70)^{bB}	3.08 (1.75)^{b}
	Mean	2.97 (1.72)^{B}	3.07 (1.75)^{B}	3.33 (1.83)^{A}	3.37 (1.83)^{A}	2.97 (1.72)^{B}	
Egg eclosion (%)	Foliage	84.00 (55.14)^{bA}	85.33 (55.52)^{bA}	78.00 (51.96)^{bB}	70.67 (49.25)^{cB}	88.00 (56.77)^{cA}	81.20 (53.73)^{b}
	Squares	88.67 (57.04)^{bAB}	88.67 (57.01)^{bAB}	81.33 (53.78)^{bBC}	78.67 (52.71)^{bC}	92.00 (59.85)^{bA}	85.87 (55.99)^{b}
	Bolls	91.33 (72.88)^{aA}	90.67 (73.21)^{aA}	86.67 (68.66)^{aB}	82.00 (65.30)^{aB}	92.00 (73.58)^{aA}	88.53 (70.73)^{a}
	Mean	88.00 (61.69)^{A}	88.22 (61.91)^{A}	82.00 (58.13)^{B}	77.11 (55.61)^{B}	90.67 (63.40)^{A}	

*Based on examination of 50 eggs in three replicates obtained randomly from egg laying of five moths. Figures in parentheses of first parameter are square root transformed values and second parameter are arc sine transformed values. Means in a column/row followed by same small/capital letter(s) respectively are not significantly different ($P = 0.05$) by DMRT.

and low in K 11 (0.050). RCH 2 recorded the highest suitability index (2.425) and the lowest was on K 11 (1.837) for the growth and development of *H. armigera* (Table 7).

DISCUSSION

Larval food plays an important role in the establishment of insect population on a plant surface. These determine the build up of population by influencing growth and development during larval stage and longevity and fecundity during adult stage (Saxena, 1969). Early instars of *H. armigera* feed exclusively on the foliage of host plants while later instars bore into pods and fruits. The present investigation elucidates the effects of various plant parts (foliage, squares and bolls) of different cotton varieties or hybrid on development of larval and pupal stages, the life cycle and various growth indices of *H. armigera*.

The larval and pupal periods showed significant differences and they were high when the larvae reared on foliage and low on bolls. This is in consonance with Kumar *et al.* (1995). Earlier studies (Doss, 1979; Bilapate, 1988; Goyal and Rathore, 1988) under varying environmental conditions the larval period was lower on bolls than on foliage. On the other hand, larval and pupal periods have shown significant differences

TABLE 6. Per cent pupation, adult emergence and developmental period of *H. armigera* larvae reared on different plant parts of selected cotton varieties/hybrid

Biological parameters*	Plant part	Varieties/hybrid					
		LRA 5166	SVPR 2	KC 2	K 11	RCH 2 (Hybrid)	Mean
Pupation (%)	Foliage	65.00 (59.10)^{aAB}	63.33 (60.11)^{aAB}	58.33 (57.84)^{aBC}	50.00 (54.83)^{aC}	70.00 (61.17)^{aA}	61.33 (58.61)^{a}
	Squares	70.00 (56.80)^{aA}	71.67 (57.85)^{aA}	61.67 (51.81)^{bB}	56.67 (48.84)^{bB}	73.33 (58.97)^{abA}	66.67 (54.85)^{b}
	Bolls	73.33 (53.85)^{bAB}	75.00 (52.76)^{bBC}	71.67 (49.80)^{bC}	66.67 (45.00)^{cD}	76.67 (56.79)^{bA}	72.67 (51.64)^{c}
	Mean	69.44 (56.58)^{A}	70.00 (56.91)^{A}	63.89 (53.15)^{B}	57.78 (47.56)^{B}	73.33 (58.98)^{A}	
Adult emergence (%)	Foliage	73.33 (60.24)^{aA}	76.67 (61.24)^{bA}	63.33 (55.76)^{aBC}	66.67 (53.81)^{aC}	76.67 (58.94)^{bAB}	71.33 (58.00)^{a}
	Squares	71.67 (57.85)^{aB}	83.33 (65.91)^{aA}	66.67 (54.84)^{abBC}	65.00 (53.74)^{aC}	83.33 (66.10)^{aA}	74.00 (59.69)^{a}
	Bolls	75.00 (59.21)^{aA}	76.67 (61.24)^{bA}	68.33 (52.73)^{bB}	65.00 (54.84)^{aB}	73.33 (61.12)^{bA}	71.67 (57.82)^{a}
	Mean	73.33 (59.10)^{B}	78.89 (62.80)^{A}	66.11 (54.44)^{C}	65.56 (54.12)^{C}	77.78 (62.05)^{AB}	
Develo-pmental period	Foliage	39.1 (6.25)^{aB}	39.7 (6.30)^{aAB}	41.7 (6.46)^{aAB}	42.7 (6.53)^{aA}	38.9 (6.24)^{aB}	40.44 (6.36)^{a}
	Squares	36.1 (6.01)^{bB}	36.8 (6.07)^{bB}	38.6 (6.21)^{aAB}	40.5 (6.36)^{aA}	36.5 (6.04)^{bB}	37.70 (6.14)^{b}
	Bolls	32.3 (5.68)^{cB}	32.9 (5.74)^{cB}	34.7 (5.86)^{cAB}	36.6 (6.05)^{bA}	32.3 (5.70)^{cB}	33.80 (5.81)^{c}
	Mean	35.83 (5.98)^{B}	36.47 (6.03)^{B}	38.33 (6.19)^{AB}	39.93 (6.32)^{A}	35.97 (5.99)^{B}	

*Mean of three replications based on 5 observations. Figures in parentheses of first and second parameters are arc sine transformed values and third parameter is square root transformed values. Means in a column/row followed by same small/capital letter(s) respectively are not significantly different ($P = 0.05$) by DMRT.

among the varieties/hybrid. This is in contrary with Kumar *et al.* (1995) who found no significant differences among cultivars tested by them in this regard. The larval and pupal periods were significantly higher in K 11 variety with 21.07 and 15.50 days, respectively in the present study.

The weight of larvae and pupae were higher on bolls and lower on foliage. This is also in accordance with Kumar *et al.* (1995). However, they did not find any significant differences among the varieties/hybrid in this aspect. The maximum larval and pupal periods and minimum larval and pupal weights recorded in K 11 may be due to the presence of some secondary substances in that variety, which would have delayed the development and reduced the weight. In general, the foliage may not have adequate

TABLE 7. Indices for the development of *H. armigera* on different cotton varieties/hybrid

Variety/hybrid	Growth index	Howe's growth index	Suitability index
LRA 5166	4.526	0.058	2.292
SVPR 2	4.573	0.057	2.315
KC 2	4.162	0.053	2.108
K 11	3.623	0.050	1.837
RCH 2 (Hybrid)	4.792	0.058	2.425

$$\text{Growth index} = \frac{\text{Mean per cent pupation (\%)}}{\text{Mean larval period (days)}}$$

$$\text{Howe's growth index} = \frac{\text{Log adult emergence (\%)}}{\text{Mean developmental period (days)}}$$

$$\text{Suitability index} = \frac{\text{Sum of all indices}}{2}.$$

nutrients compared to squares and bolls for the development of larvae, pupae which resulted in higher larval and pupal periods and lower larval and pupal weights.

The mean successful pupation (%) varied significantly among varieties/hybrid as well as in different parts of the plant. It was lower on KC 2 and K 11 varieties compared to other varieties/hybrid. Interestingly, it was noticed that when the larvae reared on foliage, the per cent pupation was high. The neonate larvae could feed only foliage because of the softness of foliage and they could not bore into bolls or squares. Though the foliage had inadequacy of nutrients, the larvae survived with delayed development, hence, the survival rate increased on foliage than on bolls and squares. Sobrindo *et al.* (1991) reported that the highest mortality rate occurred in first instar larvae fed on bolls. The same result was also obtained by Kumar *et al.* (1995). The adult emergence was not influenced by larval feeding on different parts of the plant. However, there were significant differences observed among varieties/hybrid for adult emergence. The lowest adult emergence was observed in K 11 followed by KC 2 with 65.56 and 66.11 per cent, respectively. The highest adult emergence (78.89%) was recorded on SVPR 2.

The pre-oviposition, oviposition and post-oviposition periods significantly varied for different parts of the plant as well as among the varieties/hybrid. Pre-oviposition period was higher for foliage reared larvae and lower for bolls reared larvae whereas the oviposition and post-oviposition periods were higher for larvae reared on bolls than on squares and foliage. In contrary to this, Goyal and Rathore (1988); Kumar *et al.* (1995) reported that *H. armigera* larvae reared on different parts of cotton plant did not significantly influence the pre-oviposition and post-oviposition periods. Kumar *et al.* (1995) also concluded that varieties tested by them showed non significant differences in this regard. The pre-oviposition period was relatively maximum for K 11 and KC 2 varieties, however, oviposition and post-oviposition periods were minimum for those varieties. The other varieties performed inversely in this aspect.

The fecundity of *H. armigera* moths obtained from larvae reared on different plant parts was higher on bolls (697.32 eggs) and squares (670.48 eggs) than on foliage (581.78 eggs). Under varying environmental conditions, the fecundity of *H. armigera* was higher on the squares (Bilapate, 1988) and on bolls (Doss, 1979), which indicated that moths lay more eggs when the larvae were reared on reproductive plant parts than on vegetative plant parts. As the larvae could have derived sufficient nutrients from squares and bolls compared to that of foliage. The fecundity is higher on squares and bolls than on foliage. This is in accordance with Kumar *et al.* (1995), however, they found no significant differences among the cultivars tested. K 11 and KC 2 varieties recorded (581.93 and 618.07 eggs) lower fecundity compared to other varieties. This is probably because of inadequacy of nutrients and presence of antinutritional substances in those varieties.

The adult longevity of male and female were higher when larvae reared on bolls followed by squares and foliage. According to Goyal and Rathore (1988) and Kumar *et al.* (1995), adult longevity of male was not affected by different parts of the plant and significant differences were observed for female longevity only. In the present study, significant differences were observed in adult longevity of male and female among the varieties/hybrid. The male and female longevity were lower when the larvae reared on K 11 and KC 2 varieties. Sison and Shanower (1994) observed significant differences in the longevity of *H. armigera* moths, reared on different genotypes of pigeonpea.

The incubation period was higher when the larvae reared on squares than on bolls and foliage. But Kumar *et al.* (1995) opined that incubation period was higher on both squares and foliage than on bolls, however, they found no significant differences among the varieties/hybrid regarding this aspect. In the present study, incubation period was higher on K 11 and KC 2 varieties compared to other varieties/hybrid. The egg eclosion was higher when larvae reared on bolls (88.53%) followed by squares (85.87%) and foliage (81.20%). Similar results were obtained by Bilapate (1988), Goyal and Rathore (1988) and Kumar *et al.* (1995). K 11 and KC 2 showed minimum egg eclosion compared to other varieties/hybrid.

The developmental period was significantly higher when the larvae reared on foliage (40.44 days) followed by squares (37.70 days) and bolls (33.80 days). These results corroborate with the reports of Kumar *et al.* (1995). As the insect completed its development in a shorter time on bolls, it seems that bolls are ideal food for *H. armigera* for development. This is probably due to higher nutritive value in this plant part (Bilapate, 1988; Goyal and Rathore, 1988).

Lower growth index, Howe's growth index and suitability index were recorded in K 11 and KC 2 indicating their less suitability for the growth and development of *H. armigera*. The growth index for *H. armigera* on cotton bolls was 6.3 (Goyal and Rathore, 1988) and 3.3 (Kumar *et al.*, 1995).

In general, *arboreum* type recorded longer larval, pupal, pre-oviposition, incubation, developmental periods, shorter oviposition and post-oviposition period and low larval and pupal weight, fecundity, adult longevity, per cent eclosion, pupation and adult emergence compared to *hirsutum* hybrid. The significant variations in larval pe-

riod and weight, pupal period and weight, pupation, adult emergence, pre-oviposition, fecundity, longevity, incubation period and developmental period of *H. armigera* on various parts of the cultivars/hybrid suggest differences in the nutritional and biochemical constituents. However, further study is needed to isolate and characterize these factors, which will help to understand the interactions of its establishment on the plant surface/various parts of the plant.

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A new species of thrips (Thysanoptera) of the genus *Holurothrips* Bagnall from Manipur, NE India

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ABSTRACT: Fungal spore feeding idolothripine, *Holurothrips manipurensis* sp. nov. (Phlaeothripidae : Tubulifera : Thysanoptera), collected from the oak leaf litter habitat of Manipur is described. Occurrence of this genus in India is known only through this species.

KEYWORDS: Mycophagous thrips, Thysanoptera, oak leaf litter, *Holurothrips manipurensis*

INTRODUCTION

The genus *Holurothrips* of the subfamily Idolothripinae includes spore feeding thrips that inhabit leaf litters. *Holurothrips* is related to the Ethiopian genus *Hystricothrips* Karny and therefore it is placed in the Hystricothripina (Mound, 1974). It also shows resemblance with the Oriental genera *Neatractothrips* and *Paractinothrips* (Mound and Palmer, 1983). Three species are known under the genus *Holurothrips* from Indonesia, Japan and Queensland and they are *H. ornatus* Bagnall, *H. morikawai* Kurosawa and *H. collessi* Mound respectively. But the occurrence of *Holurothrips* is not known so far from the Indian subcontinent (Ananthakrishnan and Sen, 1980) and hence, the present report is first of its kind. The following account gives the description of *Holurothrips manipurensis* sp. nov collected from Manipur.

Genus *Holurothrips* Bagnall

1914. *Holurothrips* Bagnall, *Ann. Mag. nat. Hist.*, (8) **14**: 375–381.
(Type–species *H. ornatus* Bagnall, by monotypy). (Source: Mound, 1974)

Head projection in front of eyes as long as or longer than dorsal length of eyes; eyes prolonged ventrally almost to posterior margin of head; 2 pairs of interocellar setae, I pair of postocellars, and 2 pairs of postoculars behind inner margin of eyes; cheeks

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with stout setae. Fore femora with 2 or more stout setae on exterior margin; fore tarsus unarmed, forewings with double fringes. Metanotum with a pair of strong setae; pelta broad, trilobed. Tube longer than head.

Holurothrips manipurensis sp. nov. (Fig. 1)

Macropterous female

Head dark brown, rest of the body yellowish brown, antennal segment-I dark brown, II yellowish brown, III yellow, IV and V yellow at basal third faintly shaded at apex, VI–VIII yellowish brown to dark brown. Major setae on head, thorax and leg not so brown but with broadly expanded apices. Forewing faintly shaded at apex.

Head longer than wide, dorsal surface of head largely obscured due to dense pigment, eyes prolonged little ventrally at posterior margin of head; maxillary stylets broad 'V' shaped. Pronotal anteroangular setae longer than anteromarginals and midlaterals, mesoeusternum with about 40 pairs of setae, metanotum with a pair of well developed setae (90–135 long), fore femora with 2 or more stout setae (96 long) on exterior margin, fore tarsus unarmed. Forewing with 21 double fringes; pelta broad, trilobed with apex pointed. Tergite II with a pair of sigmoid setae, III–VII with 3 pairs of wing retaining setae, VIII and IX with reticulated dorsal surface sculpture. Tube 6 times as long as tergite IX and more than twice as long as head; tube with a few weak setae.

Body measurements

Head: 632–703 long, 280–307 wide across eyes, 271–298 across cheeks, 252–289 at base. Head projection 289–315 long; 115–140 wide. Eyes 110–126 long. Ocellar setae 85–130, postocular 95.

Antennal segments length (width)

I: 94–105(68–74); II: 63–85(47–58); III: 577–610(31–42); IV: 304–336(31–36); V: 262–294(31–42); VI: 168–189(26–31); VII: 105–126(21); VIII: 105–126(21).

Antennal segments I and II with stout dorsal setae (50–80 long), IV with 2 sense cones 70–90 long, Mouth cone broad, 105–199 long, 252 wide at base, 105 at apex.

Prothorax 262–283 long, 262 wide across anterior margin, 420–462 at middle, 273–315 across posterior margin; Prothoracic setae: anteroangulars 78–105, antero-marginals 21–42, midlaterals 52, post angulars 52–63, epimerals 78–105 long. Mesothorax 105–189 long, 525–703 wide. Metathorax 315–430 long, 630–756 wide. Fore femora 388–420 long, 105–126 wide at base. Forewings 1900–1921 long, 105 wide at middle, 73–84 at base and 42–52 at apex; sub-basal setae 52–68, 130, 250–280.

Abdomen tergite IX: 579–778 wide at base; 560–832 at middle, 250–300 long. B1, B2 and B3 setae of tergite IX 190–250; 108 and 90 long respectively. Tube 1600–2000 long, 84–105 wide at base, 52–94 at middle, 52–73 at apex; 110–1260 long. (All measurements in μ.)

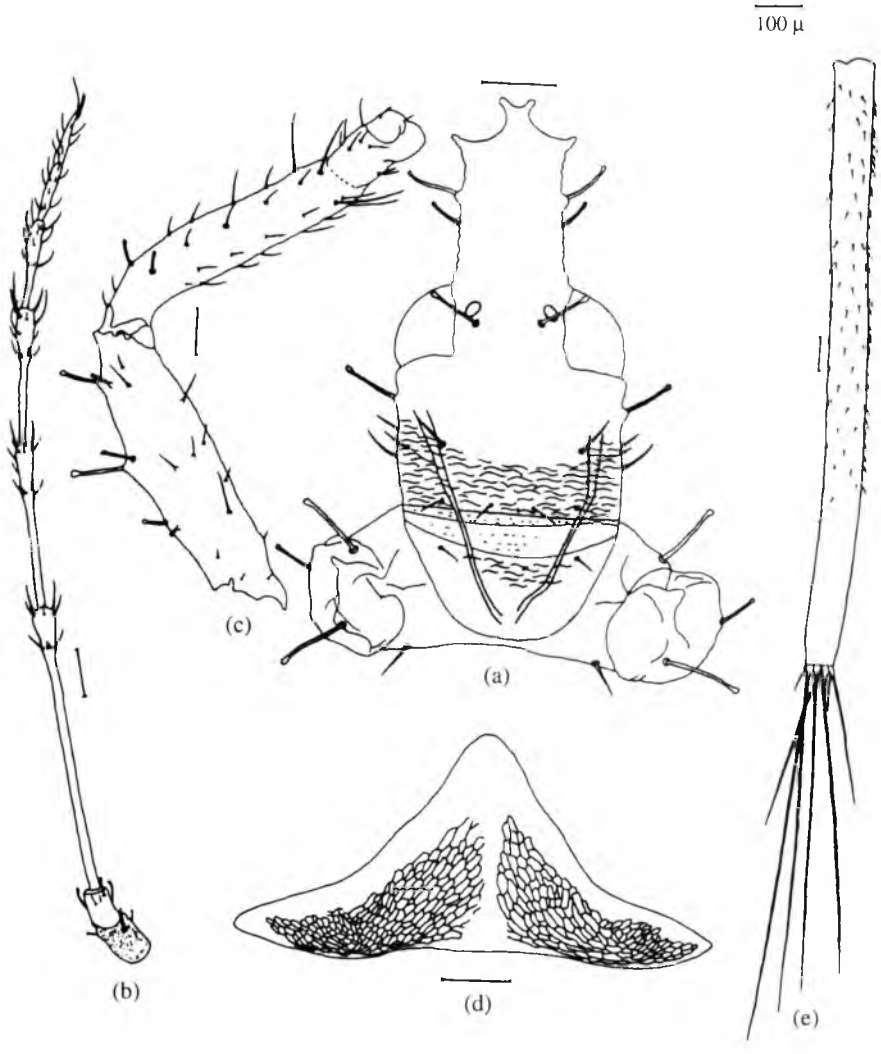

FIGURE 1. *Holurothrips manipurensis* sp. nov. (a) Head and Pronotum; (b) Antenna; (c) Fore leg; (d) Pelta; (e) Tube.

Total body length: 5.22–6.45 mm

Holotype ♀ (alate), M.U. Insect Collection Record No. 34H, Litan, Ukhrul District, Manipur State, 1500 m MSL, Ex. Oak leaf litter, 17.xi.2000, Coll. R. Varatharajan.

Paratype

2♀♀ (alate & apterous-one each), M.U. Insect Collection Record No. 34P. Rest of the data same as holotype.

Other collections (a) 2♀♀ (alate & apterous-one each), 1♂, M.U. Insect Collection Record No. 35, Tamenglong District, Manipur State, 1250 m MSL, Ex. Oak and Bamboo leaf litter, 15.x.2001.

(b) 1♀ (alate form), M.U. Insect Collection Record No. 36, Senapati District, Manipur State, 950 m MSL. Ex. Oak leaf litter, 10.xii.2001.

Etymology

As this new species has been collected from at least three different districts of Manipur, it is named as *Holurothrips manipurensis*.

Remarks

The new species *H. manipurensis* is similar to *H. ornatus* for the characters such as body colour, stout setae on head with expanded apices, anteroangulars longer than mid laterals, 3 pairs of wing retaining setae on tergites III–VII and tube < twice as long as head, but different from *H. ornatus* in terms of number of setae in mesoeusternum, length of dorsal setae in antennal segment I, position of interocellar setae and tube length in comparison to tergite IX.

H. manipurensis also exhibits similarity with *H. collessi* in terms of antennal colouration, presence of sigmoid setae on tergite II, 3 pairs of wing retaining setae on tergites III–VII and broad mesopraesternum. However, *H. collessi* is easily distinguishable from *H. manipurensis* by having short anteroangular setae and 4 sense cones on antennal segment IV, 46 double fringes, besides tube ¡ twice as long as head. In short, the long tube and about 40 pairs of setae in mesoeusternum distinctly differentiate *H. manipurensis* from other species of *Holurothrips*. The description of the Japanese species, *H. morikawai* has not been referred and compared in the present study. But Mound and Palmer (1983) distinguished *H. ornatus* from *H. morikawai* on the basis of difference in number of wing retaining setae on tergites IV–VI. The following key further substantiates the morphological variations observed among the 4 species.

KEY TO THE SPECIES OF *HOLUROTHRIPS*

1. Two pairs of wing retaining setae on tergites IV–VI ..... *morikawai* Kurosawa, 1968
– 3 or > 3 pairs of wing retaining setae on tergites IV–VI 2
2. Antennal segment IV with 4 sense cones; pronotal anteroangular setae longer than midlaterals, mesoeusternum with 30 pairs of setae, tube 1.5 times as long as head
..... *collessi* Mound (1974).
– Antennal segment IV with 2 sense cones; pronotal anteroangular setae shorter than midlaterals, mesoeusternum with > 30 pairs of setae, tube more than twice as long as head 3

3. Dorsal setae in antennal segment I short, 2 pairs of interocellar setae on head projection placed almost in the same line. Width ratio of head projection, head & pronotum 1:2:3, mesoeusternum with > 60 pairs of setae. Tube 3 times longer than tergite IX ..... *ornatus* Bagnall, 1914
– Dorsal setae in antennal segment I long, 2 pairs of interocellar setae on head projection placed wide apart. Width ratio of head projection, head and pronotum 1 : 3 : 4, mesoeusternum with about 40 pairs of setae. Tube 6 times longer than tergite IX ..... *manipurensis* sp. nov.

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Storage protein profiles in multivoltine silkworm breeds of *Bombyx mori* (L).

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ABSTRACT: Storage protein variation was studied in eleven popular multivoltine silkworm (*Bombyx mori*) breeds encompassing different parentage and origin. The main feature of storage protein variation in the multivoltines is an inter origin variability in unit area expression of storage protein. The storage protein levels (SP-2) among these popular breeds as seen in SDS-PAGE also differed. Based on densitometry scanning of SDS-PAGE bands of storage protein levels, six clustering of eleven races was done using Ward's Minimum Variance clustering analysis. Storage protein has an important role to play on metamorphic features of larval weight and pupation rate of *B. mori* as evident from correlation factors. Storage protein level in silkworm breeds and its effect on metamorphosis is discussed.

KEYWORDS: Storage protein, *Bombyx mori*, multivoltine

INTRODUCTION

Insect haemolymph proteins attracted a great deal of attention as a biochemical model system (Wyatt and Pan, 1978; Law and Wells, 1989). The main haemolymph proteins, lipophorin (LP), storage protein (SP) and vitellogenin (Vg) are common in insects and have special function for development, metamorphosis and reproduction. Storage proteins are the major reservoirs for amino acids that are utilized for cuticular proteins and for adult development (Levenbook, 1985). The development of electrophoretic procedure permits rapid and reliable identification of protein variation patterns reflecting single genetic difference (Smithies, 1955; Hunter and Market, 1957).

In lepidopterans, storage protein is synthesized by the fat body during the final instar and released into the haemolymph. Accumulation of storage protein is greatly

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influenced by genetic factors like breed and race of test individuals (Hanumappa and Delvi, 1989). Various authors have employed different procedures to estimate the amount of a particular insect storage protein. Munn and Greville (1969) used ultra centrifugation data for quantification of storage protein-I and Kinnear and Thomson (1975) relied on visual inspection of stained PAGE bands for gross appraisal of the various storage proteins, while Sekaris and Scheller (1977) estimated storage protein-I by densitometry of SDS-PAGE gels and still others, *viz.*, Tojo *et al.* (1978, 1980) employed quantitative immunodiffusion to estimate the relative concentration of storage protein-I and II of *Hyalophora cecropia* and *Bombyx mori*. Storage protein is first detectable at different stages of larval growth depending upon species but their titer increases dramatically during the last larval instar of all species. There are several reports on storage protein profiles during different larval stages of lepidopterous insects (Levenbook, 1985). However, hardly any work has been published on the variability of protein expression in germplasm stocks of silkworm breeds to qualitatively and quantitatively classify/group the silkworm stocks. This present paper focuses on the variability in the expression of storage protein synthesis in various multivoltine silkworm breeds of different parentage and origin for possible clustering in the germplasm bank.

MATERIALS AND METHODS

The silkworms of eleven multivoltine breeds were reared in the rearing house of Germplasm Centre at Hosur, Tamil Nadu, India (latitude 12°45′N and longitude 77°5′E, altitude 942 M). The standard rearing techniques were followed. All the larvae were fed on mulberry leaves of one variety and reared under controlled temperature and humidity (Krishnaswamy, 1978). Ten Vth instar, four day old, larvae were randomly collected and were bled at the distal region of the prothoracic leg and the haemolymph was drawn into an Eppendorf tube kept in ice with phenylthiourea. The mixtures were centrifuged at 12000 rpm for 5 min to remove the haemocytes and other tissue debris. The haemolymph samples collected were stored at −20°C.

After quantification of protein (Bradford, 1976), haemolymph extracts equivalent 50 μg of protein were mixed with equal volume of SDS-sample buffer, and the mixture were boiled for 1 min and subjected to SDS-PAGE under 6% stacking gel and 7.5% separating gel (Laemmli, 1970). Molecular weight of polypeptides was compared with standards already available at Dept of Biotechnology, Bharathisdasan University, Trichy, Tamil Nadu, India. Densities of protein bands were measured with a densitometry scanner (Alfa, Tech, USA). SP-2 protein was identified by its position in the gel. Clustering analysis of the results based on unit area of expression of storage protein was made using Ward's Minimum Variance Clustering analysis. Correlation analysis between metamorphic features and storage protein was done using SPSS package.

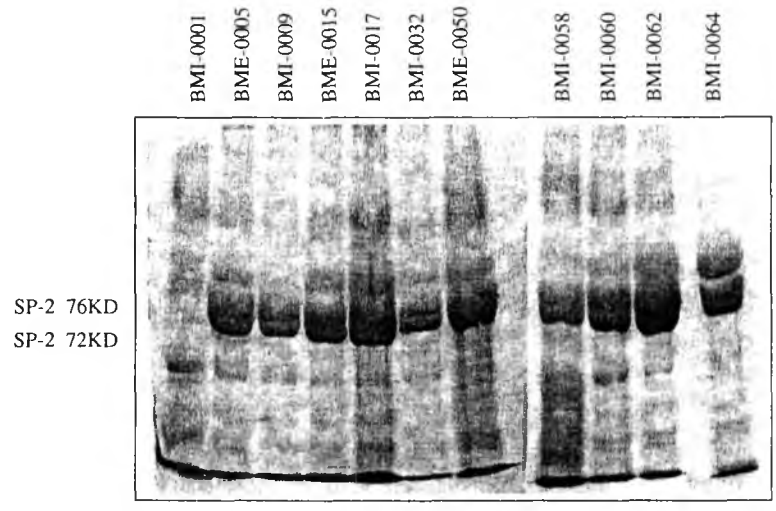

FIGURE 1. Variability in storage protein expression among eleven multivoltine silkworm breeds.

RESULTS

The haemolymph storage protein profile of eleven multivoltine breeds of different origin and percentage of *B. mori* is shown in Fig. 1. The staining intensity of storage protein SP-2 a major haemolymph protein in insects having a molecular range of 72–76 KD, was found varying in eleven breeds investigated (Table 1). Based on the storage protein synthesis, relationship among the selected eleven multivoltine silkworm breeds was studied. A dendrogram generated by hierarchical clustering technique is shown in Fig. 2. Cluster analysis is a standard method to study relatedness and genetic diversity among breeds. Eleven multivoltine silkworm breeds were resolved into six clusters based on storage protein expression. The West Bengal origin breed BMI-0032 and Karnataka origin breed BMI-0009 get clustered under one group. It is clear from the dendrogram that the breeds BMI-0017 and BMI-0001 are the most divergent in their ability to store amino acids as energy source.

The highest unit area of expression of storage protein SP-2 was recorded in breed BMI-0017 (Nistari) and lowest expression in BMI-0001 (Pure Mysore) (Table 1). Dendrogram analysis also indicated that these two breeds separately stands as distinct groups (Fig. 2). Pure Mysore breed BMI-0001, a locally originated breed adopted to tropical conditions which showed the lowest storage protein level of 624 units and a minimum of economic characters of lower larval weight of 18.932 g, slower developmental rate of 0.0355 and lower pupation rate of 55.54%, when compared over other breeds were selected for this study (Table 2).

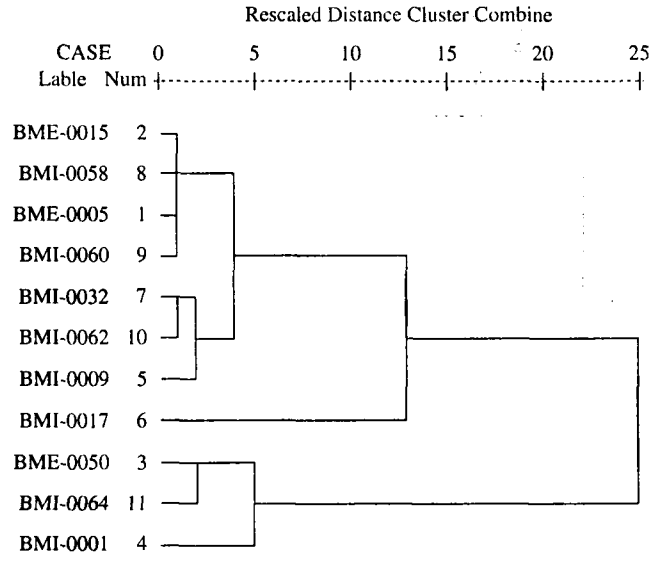

FIGURE 2. Dendrogram of 11 multivoltine silkwork accessions based on storage protein.

TABLE 1. Variability in protein expression of storage protein (SP-2) level in multivoltine silkworm breeds of *B. mori*

Sl. No.	Multivoltine breeds	Name of breed	Parentage	Origin	Storage protein type	Peak Numbers of SP-2	Unit area of SP-2 expression
1.	BMI-0001	Pure Mysore	Original race	KAR	SP-2	6, 7	624
2.	BME-0005	C. Nichi	Original race	JAP	SP-2	6, 7	3722
3.	BMI-0009	Kollegal Jawan	PM. NN6 D	KAR	SP-2	5, 6	5642
4.	BME-0015	Raj	Original race	BGD	SP-2	7, 8	4094
5.	BMI-0017	Nistari	Original race	WBL	SP2	10, 11, 12	6649
6.	BMI-0032	A4e	Mys1.BW (Mys (N122, C120) (N.124, C124)	WBL	SP2	7, 8	4737
7.	BME-0050	Cambodg	Original race	JAP	SP2	10	1553
8.	BMI-0058	BL23	—	KAR	SP2	2, 3	3996
9.	BMI-0060	MU303	—	KAR	SP2	6, 7	3612
10.	BMI-0062	MU10	—	KAR	SP2	5, 6	4898
11.	BMI-0064	SK6XSK1XTW	—	KAR	SP2	4	2465

Note: KAR = Karnataka, India; JAP = Japan; BGD = Bangladesh; WBL = West Bengal, India.

TABLE 2. Metamorphic features of eleven multivoltine breeds of silkworm *B. mori*

Sl. No.	Multivoltine breeds	Name of breeds	Larval weight (g/10 larvae)	Pupation rate (%)	SP-2 level (units)
1	BMI-0001	Pure Mysore	18.932	55.540	624
2	BME-0005	C. Nichi	22.013	76.410	3722
3	BMI-0009	Kollegal Jawan	32.057	76.740	5642
4	BME-0015	Raj	23.671	73.450	4094
5	BMI-0017	Nistari	25.353	76.150	6649
6	BMI-0032	A4e	30.006	77.780	4737
7	BME-0050	Cambodg	24.772	72.540	1553
8	BMI-0058	BL23	29.304	65.990	3996
9	BMI-0060	MU303	29.444	75.670	3612
10	BMI-0062	MU10	27.869	81.700	4898
11	BMI-0064	SK6XSK1XTW	17.038	71.240	2465

TABLE 3. Coefficient of correlation between metamorphic features and storage protein

	Developmental rate	Larval weight	Pupation rate	SP-2
Developmental rate	—	0.229	0.586	0.455
Larval weight		—	0.494	0.620*
Pupation rate			—	0.701*
SP-2				—

Note: *Correlation significant at 5% level.

DISCUSSION

The studies on genetic divergence of multivoltine silkworms (*B. mori*) and clustering pattern of 22 multivoltine silkworm based on Mahalanobis D2 analysis also indicate the accession BMI-0001 (Pure Mysore) as a distinct cluster from all other accessions (Kumaresan *et al.*, 2003).

Correlation values and ranking sequences (Tables 3 and 4) also indicated a significant relationship between storage protein and metamorphic features, *viz.*, larval weight and pupation rate. The main haemolymph protein, namely storage protein, has a special function for metamorphosis in insects (Levenbook, 1985).

In lepidopteran insects, synthesis of the storage protein (SP-2) is regulated by juvenile hormone (JH) suggesting that the regulation of storage protein synthesis by JH differs between species/races (Chinzei *et al.*, 1994). Exogenous juvenile hormone application in rice moth *Corcyra cephalonica* decreased the haemolymph storage protein levels suggesting that JH level alters the expression of storage protein (Ismail and Dutta-Gupta, 1988). Further, most of the development processes in insects is directly or indirectly are being controlled by hormones (Calvez, 1981). The observed

TABLE 4. Ranking sequence of storage protein (SP-2) and metamorphic features *viz.* larval weight (Lwt), pupation rate (Pup rate) and developmental rate (DR)

Sl. No.	Multivoltine breeds	Name of breeds	SP-2	Lwt.*	Pup rate*	DR
1	BMI-0001	Pure Mysore	11	10	11	8
2	BME-0005	C. Nichi	7	9	4	3
3	BMI-0009	Kollegal Jawan	2	1	3	5
4	BMI-0015	Raj	5	8	7	7
5	BMI-0017	Nistari	1	6	5	4
6	BMI-0032	A4e	4	2	2	6
7	BME-0050	Cambodg	10	7	8	5
8	BME-0058	BL23	6	4	10	2
9	BMI-0060	MU303	8	3	6	4
10	BMI-0062	MU10	3	5	1	4
11	BMI-0064	SK6XSK1XTW	9	11	9	1

Note: *Close relationship with storage protein based on ranking sequence of metamorphic features correlation is significant at 5% level.

variability in the synthesis of storage protein in the present study may be attributed to the difference in JH regulation in silkworm breeds investigated.

Densitometry scanning of bands relating storage proteins exhibit wide variations in the staining intensity and this may be related to differential regulatory pattern by juvenile hormone (JH) of these accessions. Apart from this, the staining intensity of SP-2 bands also differed much among origin and parentage. Japanese and Bangladesh origin breed BME-0005 and BME-0015 established a close relationship with Karnataka origin BMI-0060 indicating close affinity in pattern of protein expression. Further studies on storage protein in various silkworm breeds may throw light on the regulatory pattern of protein expression relating to metamorphosis and development. The present investigation leads us to conclude that storage protein, a major haemolymph protein secreted by fat body of silkworm, has an important role to play on metamorphic features, *viz.*, larval weight and pupation rate in the lepidopteran insect, *B. mori* and this may be considered as a dependable biochemical index by breeders and researchers for selecting the reeds for further multiplication of new breed/lines.

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Influence of the leaf, flower and pod extracts of *Moringa oleifera* on the growth and reproductive parameters of *Bombyx mori* L.

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ABSTRACT: Dietary supplementation of the leaf, flower and pod extracts of *Moringa oleifera* (1% W/V) elicited varied responses in the final instar larvae of the mulberry silkworm, *Bombyx mori*. The mean larval weight and the weight and size of cocoon increased significantly as a result of this supplementation. Although, the weight of the male and female pupae increased considerably, the increase in female pupae was much higher in the case of *Moringa* leaf extract supplementation and this was reflected in the weight of their ovariole also. Significant increase in the weight of male and female moths was noticed in the experimental sets that received the leaf extract supplementation. The number and weight of the eggs were found to increase significantly in the sets that received the dietary supplementation of *Moringa* leaf, flower and pod extracts. However, the protein content of the eggs increased to a large extent only in the sets that received the *Moringa* leaf extract. The advantages of the dietary supplementation with Moringa leaf, flower and pod extracts in improving the growth and reproductive potential of the silkworm are discussed.

KEYWORDS: Silkworm, nutrition, *Moringa*, growth and reproduction

INTRODUCTION

Plants are the richest source of organic chemicals on earth and the phytochemicals have been reported to influence the life and behaviour of different insects (Rajashekharagouda *et al.*, 1997). Murugan *et al.* (1998) reported the growth promoting effect of the aqueous extracts of some plants such as *Tribulus terrestris, Phyllanthus niruri, Boerhavia diffusa, Psoralea corylifolia, Caesalpinia coriaria and Parthenium hysteophorous* in the silkworm, *Bombyx mori*. They observed changes in the electrophoretic protein patterns of the silk gland along with the increase in the level of proteins. Reserpine, an alkaloid isolated from the roots of *Rauwolfia serpentina* has

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been reported to act as a neurotransmitter influencing the metabolism of glycogen and trehalose during the fifth instar of *B. mori* (Sujatha and Rao, 2002). This paper reports the influence of dietary supplementation of the leaf, flower and pod extracts of *Moringa oleifera* on the growth and reproductive parameters of the silkworm, *B. mori* L.

MATERIALS AND METHODS

The leaves, flowers and pods of *M. oleifera* were collected and shade dried separately. They were then blended in a mixer grinder to a fine powder. Ten grams each of the leaf, flower and pod powder were weighed out and packed separately in burettes keeping glass wool at the bottom. Warm acetone was then poured on top of the burette column and the resultant extracts were collected until clear acetone trickled down. The extracts were concentrated by evaporating acetone in a hot water bath and made up to 10 ml with distilled water. For the present study, one ml each of the extracts were diluted to 100 ml with distilled water to get 1% concentration and used as test solutions. Disease free layings of the silkworm race $PMxNB_4D_2$ were obtained and the larvae were raised on fresh mulberry leaves of MR_2 variety following standard rearing methods (Krishnaswamy, 1978). Freshly moulted fifth instar larvae were selected for the experimental rearing in rectangular plastic trays. They were divided into 15 groups of 30 larvae each so as to form five triplicates each for plain control, water control, leaf, flower and pod extract treatment respectively. These larvae were fed uniformly five times a day with untreated mulberry leaves during four feedings but differentially during one feeding after bed cleaning as follows, i.e., the larvae in the plain control were given untreated mulberry leaves, the larvae in the water control were given mulberry leaves dipped in distilled water, the larvae in the other three groups were given mulberry leaves dipped in *Moringa* leaf, flower and pod extract solution respectively. The larvae were thus reared at 28 ± 2 °C and 75 ± 10%RH for the entire duration of the fifth instar. The live weights of the larvae were found out daily during the entire period of this instar. The faecal pellets were also collected separately from each tray daily at the time of bed cleaning and dried to weight constancy. The ripe larvae were hand picked and left on separate mountages for spinning cocoon. The cocoon were harvested on the fifth day and weighed. Ten of them per replicate were cut open to find out the weight of the male and female pupae, weight of the shell and shell ratio. The remaining cocoons were kept separately. Upon emergence, the weights of the male and female moths were found out immediately. The paired moths were left undisturbed for few hours, de-coupled and the mated females were allowed individually on pre-weighed papers under inverted funnels to lay eggs. The eggs were then counted and weighed. Consumption was estimated from the weight of faeces egested following the indirect method of (Mathavan and Pandian, 1974) and the efficiencies of egg production were worked out.

TABLE 1. Daily increment in the live weight of the final instar larvae of *B. mori* in response to dietary supplementation of the leaf, flower and pod extracts of *M. oleifera*

Day	Live weight of the larva (mg)				
	Plain control	Water control	Leaf extract	Flower extract	Pod extract
0	665 ± 13	672 ± 7	672 ± 10	673 ± 18	677 ± 10
1	966 ± 18	1037 ± 57	1075 ± 35*	996 ± 12	1035 ± 68
2	1403 ± 19	1516 ± 24	1462 ± 56	1394 ± 66	1414 ± 57
3	2253 ± 153	2693 ± 119**	2397 ± 70*	2493 ± 370	1603 ± 40
4	2389 ± 19	2696 ± 11*	2811 ± 36*	2553 ± 125	2370 ± 75
5	2890 ± 147	2985 ± 95	3430 ± 238*	2878 ± 192	2762 ± 251*
Mean Larval Weight	1751 ± 7	1933 ± 50	1974 ± 53	1831 ± 10	1643 ± 37

Each value is the average of 60 larvae ± SD. *Student '*t*' value for the difference from control significant at < 0.05 level.

RESULTS AND DISCUSSION

Dietary supplementation of the leaf, flower and pod extracts of *M. oleifera* was found to exert profound influence on the growth and reproductive parameters of the mulberry silkworm *B. mori*. The daily increment in the live weight of the larvae showed significant increase in the case of *Moringa* leaf extract supplemented set (Table 1). The mean larval weight of the final instar larvae was found to the 1751 ± 7 mg/larva in the plain control and 1974 ± 53 mg/larva in the case of the larvae that received *Moringa* leaf extract as the dietary supplement. The corresponding weight in the water control was 1933 ± 50 mg larva and in the case of flower supplementation it was 1831 ± 10 mg/larva. However, the mean larval weight was found reduced in the case of the larvae that received the pod extracts as dietary supplementation as 1643 ± 37 mg live wt./larva. Eid *et al.* (1989) reported increase in the weight of the silkworm when red castor leaf extract was sprayed on leaves or by injection to fifth instar larvae of the silkworm, *Philosamia ricini* and attributed this to the increase of free amino acids, carotenoids and soluble carbohydrate in the diet. Murugan *et al.* (1998) attributed the growth promoting effect of the plant extracts to the stimulation of the biochemical process of protein synthesis. The quantity and quality of dietary protein has long been considered to be important in the insect growth. A nutritionally balanced protein diet is highly essential for the optimal growth of the silkworm. Soya protein is considered as an ideal supplement that provides high quality proteins with rich amino acids, a good source of iron and vitamins. Silkworm reared on the leaf supplemented with Soya bean flour recorded significantly higher larval weight on account of additional protein supplementation (Sundarraj *et al.*, 2000). *Moringa* tree is valued mainly for its green pods which are used as vegetable. Flower and tender leaves are eaten as potherb. All parts of the tree are considered medicinal and hence are used in the treatment of

several diseases. The leaves are rich in vitamin A and C and are considered highly nutritive (Lakshmi and Mohanarao, 1998). Therefore, the inducement of the growth of silkworm in the present study may be attributed to the nutritive value of *Moringa*.

The plant extracts are also considered as the cheap and abundant source of insect hormones to increase the silk yield. Studies conducted in this respect revealed that the extracts of some of the commonly occurring weed plants of South India such as *Tribulus terrestris* and *Psoralea coryleifolia* could benefit sericulture for improving the silk and egg yield of *B. mori* for commercial silk and basic seed production (Rajashekharagouda *et al.*, 1997). In the present study, the weight and size of the cocoons were found to increase significantly over the control in the experimental sets (Table 2). Subburathinam *et al.* (1990) observed that enrichment of mulberry leaves with calcium chloride to increase the cocoon characters like cocoon weight, shell weight, cocoon/shell ratio and silk proteins. Murugan *et al.* (1998) noticed that the silkworm larvae fed on mulberry leaves supplemented with the plant extracts *Tribulus terrestris, Boerhavia diffusa and phylanthus niruri* to produce more silk.

Pupal weight is an indirect measure of the reproductive potential in insects. The weight of the pupae of the both male and female in the present study showed significant improvement over the control with the Gonado Somatic Index of the female very high at 42.04±3.22 percent as a result of the treatment with *Moringa* leaf extracts (Table 2). Mathavan *et al.* (1984) obtained increased pupal weight as a result of the supplementation of single cell protein to the final instar larvae of the *B. mori*. Rahman *et al.* (1978) had reported the influence of fortification agents to increase the pupal weight of *B. mori* and noticed that the female adults emerging from healthy and larger sized pupae tend to lay more eggs than those from smaller size pupae.

In insects with non-feeding adult stage the reproductive efficiency depends on the attainment of ideal body weight by the adults (Hinton, 1981). Thiruvalluvan and Nagendran (1999) reported that supplementation of mulberry leaves with mixture of antibiotics and vitamin showed improvement the weight of the moths and the reproductive parameters in *B. mori*. Similarly, Changamma. *et al.* (2000) also reported that the administration of juvenile hormone analogue to increase significantly the weight of male and female moths. An interesting observation in the present study is that difference between the weight of the female moths before and after egg laying was comparatively higher in the case of *Moringa* leaf extract supplementation followed by the supplementation of pod extract and flower extract. This gives an indication that an increased percentage of the accrued energy has been invested in the formation of eggs by the silkworm.

The fecundity and weight of the eggs form a direct index of the reproductive performance in insects. Heavier or larger adult insects tend to lay more eggs than smaller ones and such an increase in the number and weight of the egg would result in increased hatchability within shorter incubation period (Hinton, 1981). Magadum *et al.* (1992) reported that the treatment of 'Manta' a juvenile hormone analogue followed by thyroxine to increase the fecundity in *B. mori*. Rai *et al.* (2002) had reported significant increase in fecundity by 32.6% with folic acid supplementation

TABLE 2. Reproductive parameters observed in the silkworm, *B. mori* in response to dietary supplementation of the leaf, flower and pod extracts of *M. oleifera* during final instar

Parameter	Plain control	Water control	Leaf extract	Flower extract	Pod extract
Cocoon weight: mg wet wt./cocoon,	1303 ± 65	1333 ± 29	1366 ± 15	1288 ± 48*	1330 ± 45
mg dry wt./cocoon, Cocoon volume: (mm^3)	469 ± 23	486 ± 11	543 ± 6*	483 ± 18	558 ± 17*
	52.4 ± 8.3	63.6 ± 2.8*	68.7 ± 1.1*	65.0 ± 9.3*	61.2 ± 6.6*
Pupal weight: mg wet wt./pupa, male,	868 ± 13	892 ± 57	997 ± 22*	912 ± 12*	916 ± 56
female: mg wet wt./pupa, ovariole	1049 ± 45	1146 ± 27*	1373 ± 41*	1064 ± 87	1238 ± 117
weight: mg wet wt./pupa, gonado-somatic index:	298 ± 11	316 ± 24*	412 ± 33*	288 ± 40	295 ± 40
	38.73 ± 2.16	38.07 ± 2.16*	42.04 ± 3.22	39.94 ± 4.62	31.45 ± 3.9*
Moth weight: mg wet wt./moth, male,	387 ± 16	414 ± 16	475 ± 13*	379 ± 17	438 ± 22*
female: before egg laying, after egg laying	928 ± 27	963 ± 13	1129 ± 29*	1083 ± 10*	1045 ± 12*
	326 ± 10	357 ± 11*	364 ± 20*	383 ± 20*	349 ± 18
Fecundity: no. of egg/laying	476 ± 28	587 ± 59	625 ± 57*	520 ± 22	575 ± 14*
Egg weight: mg/laying, mg/egg	248 ± 9	303 ± 57	352 ± 13*	258 ± 20	307 ± 5*
	0.52 ± 0.02	0.55 ± 0.03	0.57 ± 0.03	0.50 ± 0.05	0.53 ± 0.01
Egg protein: (mg/fresh weight)	534	598	754	534	350
Hatching Efficiency (%)	95.2	96.7	98.7	96.6	95.6

Each value is the average of 3 to 10 observations ± SD. *Difference from the control significant at 0.05 level (Student '*t*' test).

TABLE 3. Egg production efficiency in the mulberry silkworm *B. mori* in response to dietary supplementation of the leaf, flower and pod extracts of *M. oleifera*

Parameter	Plain control	Water control	Leaf extract	Flower extract	Pod extract
C (Ng/larva)	4049	4097	3807	3883	3771
A "	1519	1537	1431	1456	1414
ECIE (%)	6.13 ± 0.22	7.38 ± 1.38 (20.4)	9.25 ± 0.34* (50.9)	6.64 ± 0.51 (8.32)	8.13 ± 0.12* (32.63)
ECDE (%)	16.30 ± 0.48	19.33 ± 3.67 (18.59)	24.62 ± 0.91* (51.04)	17.72 ± 1.38 (8.71)	21.69 ± 0.33* (33.07)
IMGE (g)	16.32 ± 0.57	13.83 ± 2.62 (−15.26)	10.81 ± 0.39* (−33.76)	15.11 ± 1.17 (−7.41)	12.29 ± 0.18* (−24.69)
DMGE (g)	6.12 ± 0.21	5.19 ± 0.98 (−15.20)	4.06 ± 0.14* (−33.66)	5.67 ± 0.43 (−7.35)	4.61 ± 0.07* (−24.67)

ECIE = Efficiency of conversion of Ingesta into eggs.
ECDE = Efficiency of conversion of digesta into eggs.
IMGE = Ingested matter per gram egg.
DMGE = Digested matter per gram egg.
The values are the averages of 3 observations ± SD; figures in parentheses indicate the percentage of change from control. *Student '*t*' values for the difference from the control significant at $\rho < 0.05$ level.

and also had suggested its role in the process of oogenesis and yolk formation in *B. mori*. In the present study, the weight of eggs/laying was found to be 248 ± 9, 303 ± 57, 352 ± 13, 258 ± 20 and 307 ± 50 mg/laying respectively with the plain control, water control and *Moringa* leaf, flower and pod extracts supplementation. The protein content of eggs also showed marked changes with the respect to the dietary supplementation of *Moringa* leaf, flower and pod extracts in the present study. For example, the protein content of the eggs in plain control was 534 mg/g at the same time it was 754 mg/g in the case of leaf extract supplementation and only 350 mg/g in the case of pod extract supplementation. This observation in the present study finds support in the results of Choudhuri *et al.* (1987) who obtained increased number of eggs as a result of thyroxine injection in *B. mori* and considered this to be due to the synthesis of female specific protein in the fat body that help in the maturation of oocytes. The reproductive success of a female is considered to depend on her efficiency in transforming ingested food energy into eggs and her ability to produce fertile offspring (Engelmann, 1970). In this context, the increased hatching efficiency observed in the present study, clearly indicates the reproductive success of the silkworm after receiving *Moringa* leaf, flower and pod extract supplementation.

Studies on animal energetics have developed quite rapidly and are widely used in predicting the probability and profitability of culturing an economically important animal. Engelmann (1966) considered this as a principle means for understanding and interpreting and complexities of nature. In the present study, the efficiency of egg production of the silkworm in response to the dietary supplementation of the leaf, flower and pod extracts of *M. oleifera* were worked out from the amount of food

consumed (C) and assimilated (A) by the silkworm in the respective sets (Table 3). The consumption was found increased from the plain control by 1.2% in water control, but decreased by 7.1, 5.2 and 8% respectively as a result of the supplementation of *Moringa* leaf, flower and pod extracts. Accordingly 1.2% increase in assimilation was noticed in the water control over the plain control and 5.8, 4.1 and 6.9% decrease were noticed with the respect to the dietary supplementation of leaf, flower and pod extracts respectively. Ito and Nakasone (1967) observed significant increase in conversion efficiencies when the larvae were fed with mulberry leaves rich in nutrients and that this increase in the conversion of efficiencies lead to an increase in the efficiency of conversion of ingested dry matter to eggs. In the present study the ECIE and ECDE were found to be maximum in the case of mulberry leaf extract supplementation registering an increase of 50.9 and 51.04% respectively over the plain control. Such an increase in the conversion efficiency of ingested and digested matter in to egg could be realized mainly because of the reduction in the dry matter requirement (IMGE and DMGE) towards the formation of eggs. These observations of the present study clearly indicate that the dietary supplementation of *Moringa* leaf, and pod extracts would benefit the sericulture industry by effective utilization of food.

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Formulation of host cadavers infected with indigenous *Heterorhabditis* spp. (Nematoda: Heterorhabditidae: Rhabditida) isolates

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ABSTRACT: Feasibility of formulating nematode-infected insect cadavers to overcome problems in storage and handling of cadavers for biocontrol of insect pests was studied using *Heterorhabditis* spp. Poinar and bioassayed on *G. mellonella* (L.). Formulation of insect cadavers by immersing in liquid suspension of cellulose and rolling in talc and kaolin had no significant deleterious effects on nematode reproduction and infectivity for one month. Four-day-old infected cadavers were more amenable to desiccation than 8-day-old cadavers. Formulated cadavers were more resistant to rupturing during storage than non-formulated cadavers.

KEYWORDS: Cadaver formulation, entomopathogenic nematodes, *Heterorhabditis indica*, *H. bacteriophora*

Entomopathogenic nematodes (EPN) (Nematoda: Heterorhabditidae) have emerged as excellent biological control agents against insect pests. Poor storage and post application survival are major obstacles to their increased usage. They are routinely stored or applied to soil for insect control in aqueous suspension (Woodring and Kaya, 1988). Several workers have attempted application of EPN in infected cadavers (Jansson *et al.*, 1993). Application of nematode infected cadavers was found better than in aqueous suspension (Shapiro and Lewis, 1999; Shapiro *et al.*, 2003). In this approach, nematode infected cadavers are disseminated and the progeny, infective juveniles (IJs) that exit subsequently achieve pest suppression. Present investigation aimed to determine the effects of various carrier materials on infected cadavers in storage.

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MATERIALS AND METHODS

The host insect Greater wax moth, *Galleria mellonella* was cultured in laboratory on artificial medium (Singh 1994) and the final instar larvae were used for the experiments. The EPN *Heterorhabditis indica* PDBC EN 13.3, *H. indica* PDBC EN 6.71, and *H. bacteriophora* originally isolated from soil samples collected from Chidambaram, Bangalore and Coimbatore respectively, were cultured *in vivo* on *G. mellonella* larvae (Woodring and Kaya, 1988). The IJs were stored in distilled water at 24 °C in tissue culture flasks @10,000–20,000 IJs/ml with intermittent aeration and change of water. Infected cadavers were produced by the exposure of *G. mellonella* last instar larvae to approximately 50 IJs in 90 mm petri dishes lined with Whatman No 1 filter paper.

The host cadavers were transferred to a petridish lined with dry filter paper 48 h after inoculation, incubated at 24 °C for 4 and 8 days and were utilized formulation as per procedure described below.

Two experiments were conduced. In the first experiment, 4 and 8 days post infection (dpi) cadavers of *G. mellonella* larvae infected with *H. indica* 13.3 were formulated by immersing in liquid suspensions of cellulose (7.5%), starch (10%), agar (2%) and gelatin (2%) and rolled in inert carrier materials *viz.*, talc (5%), kaolin (10%), bentonite (2%), until the powder uniformly adhered to its surface. The cadavers thus formulated were stored at room temperature (27 °C ± 2 °C) and tested for progeny production and pathogenicity against *G. mellonella* at weekly intervals up to 21 days. There were 20 combinations of dipping and rolling agents and nonformulated cadavers (treatments) for each 4 and 8-dpi cadavers in three replications (Table 1). The cadavers rolled in individual carrier material failed to adhere to the insect cuticle and cracked off on drying leading to shrinkage of the larval body. Hence, carriers were tried in combinations for formulation for long-term storage at ambient temperature.

In the second experiment, based on the results of first experiment where cellulose was superior with reference to the progeny production as compared to other inert materials, 4 dpi cadavers of *G. mellonella* larvae infected with the three nematode isolates were formulated and stored for one month.

Progeny production and infectivity

The reproductive efficiency of the 4 and 8-dpi formulated and non-formulated cadavers were evaluated at weekly intervals (up to 21 days) by transferring the cadavers on to a White trap (White, 1927). In each treatment, 3 cadavers (replications) were transferred on to the White trap and were examined for progeny production. The emerging IJs were collected till the emergence ceased and the number of IJs produced per cadaver was determined by dilution counts (Woodring and Kaya, 1988). Assays for infectivity were performed on *G. mellonella* as test insect. Pathogenicity of the IJs emerging at different periods after formulation were tested by exposing five *G. mellonella* larvae (50 IJs/larvae). The per cent mortality of *G. mellonella* was recorded at 24, 48 and 72 h after exposure in bioassay tests.

The data were analysed statistically using DMRT, after transforming the values.

TABLE 1. Progeny production of *H. indica* 13.3 in 4 and 8 day old formulated cadavers of *G. mellonella* in different carriers

Carrier materials	Abbreviations	No. of *H. indica* IJs from 4 and 8 d old cadavers days after (lakhs)					
		4 d-old				8 d-old	
		7	14	21	Mean	7	Mean
7.5% Cellulose + Talc	CT	3.95^{a}	3.45^{b}	1.38^{c}	2.93^{a}	2.30^{c}	0.77^{d}
7.5% Cellulose + Kaolin	CK	5.30^{a}	4.97^{a}	0.45^{d}	3.57^{a}	3.40^{b}	1.13^{d}
7.5% Cellulose + Talc + Kaolin	CTK	5.23^{a}	3.95^{a}	0.42^{d}	3.20^{a}	0.36^{d}	0.12^{d}
7.5% Cellulose + Bentonite	CB	5.00^{a}	4.40^{a}	0.58^{d}	3.33^{a}	0.86^{d}	0.29^{e}
7.5% Cellulose + Talc + Kaolin + Bentonite	CTKB	5.06^{a}	4.05^{a}	0.69a	3.26^{a}	0.48^{d}	0.16^{e}
10% Startch + Talc	ST	1.35^{c}	0.67^{d}	0.0^{d}	0.67^{d}	0.36^{d}	0.29^{e}
10% Startch + Kaolin	SK	1.95^{c}	1.23^{c}	0.45^{d}	1.09^{a}	0.69^{d}	0.31^{e}
10% Startch + Talc + Kaolin	STK	1.68^{c}	0.65^{d}	0.60^{d}	0.82^{d}	0.15^{d}	0.58^{d}
10% Startch + Bentonite	SB	4.94^{a}	4.60^{a}	1.52c	3.22^{a}	0.50^{d}	0.32^{e}
10% Startch + Talc + Kaolin + Bentonite	STKB	2.00^{c}	1.48^{c}	0.98^{d}	1.30^{c}	0.38^{d}	0.35^{e}
2% Agar + Talc	AT	2.45^{c}	1.90^{c}	0.65^{d}	1.64^{c}	2.30^{c}	0.93^{d}
2% Agar + Kaolin	AK	3.45^{b}	2.80^{b}	1.95^{c}	2.67^{b}	2.95^{b}	1.05^{d}
2% Agar + Talc + Kaolin	ATK	3.54^{b}	2.38^{c}	0.98^{d}	2.29^{b}	3.40^{b}	1.22^{c}
2% Agar + bentonite	AB	4.20^{a}	3.08^{b}	1.92^{c}	2.97^{a}	3.25^{b}	1.07^{d}
2% Agar + Talc + Kaolin + Bentonite	ATKB	3.21^{b}	2.13^{c}	0.85^{d}	2.05^{b}	2.94^{b}	0.81^{d}
2% Gelatin + Talc	GT	1.45^{c}	0.32^{d}	0.0^{d}	0.43^{d}	0.0^{d}	0.22^{e}
2% Gelatin + Kaolin	GK	1.85^{c}	0.60^{d}	0.03^{d}	0.75^{d}	1.23^{c}c	0.48^{d}
2% Gelatin + Talc + Kaolin	GTK	1.91^{c}	0.78^{d}	0.04^{d}	0.81^{d}	0.95^{d}	0.38^{e}
2% Gelatin + Bentonite	GB	1.43^{c}	0.35^{d}	0.0^{d}	0.48^{d}	0.45^{d}	0.24^{e}
2% Gelatin + Talc + Kaolin + Bentonite	GTKB	1.25^{c}	0.45^{d}	0.0^{d}	0.53^{d}	0.97^{d}	0.76^{d}
Control (non formulated)	NF	4.90^{a}	0.0^{d}	0.0^{d}	1.09^{d}	0.0^{d}	0.0^{e}

S. Em = 48. CD ($P = 0.05$) = 1.37, * Means followed by same letters are not significantly different.

RESULTS

Effects on progeny production

The reproductive efficiency of cadavers formulated in combinations of carriers CT (5.23×10^5Ijs), CK (5.3×10^5 Ijs), CTK (5×10^5 Ijs) and CB (5.06×10^5 Ijs) at 7.5% was highest seven days after storage as compared to non-formulated cadavers. The percent increase varied from 0.82 to 8.16 over the non-formulated check (4.9×10^5 Ijs). The 4-dpi formulated cadaves stored intact up to 21 days without affecting the progeny production (Table 1). The abbreviations used are explained in Table 1.

The emergence of Ijs from 8-dpi formulated cadavers was observed till seven days and no progeny production was observed 14 and 21 days after storage. The formulation materials CT and CTK at 7.5%, AT, AB, AK, ATK and ATKB at 2% were found to be effective up to seven days and yields ranged between 3.4×10^5 to 2.3×10^5 Ijs per

TABLE 2. Progeny production of *H. indica* 13.3, 6.71 and *H. bacteriophora* in *G. mellonella* from formulated cadavers stored for 1 month

Carrier material	Abbreviations	No. of IJs produced by formulated cadavers of EPN stored for one month*		
		H. indica	*H. indica*	*H. bacteriophora*
Days after post inoculation		13.3	6.71	
7.5% Cellulose + Talc + Kaolin	CTK	4.88[a]	3.98[b]	4.15[b]
7.5% Cellulose + Vermiculite + Bent	CVB	3.56[c]	0.0[k]	2.53[c]
7.5% Cellulose + Vermiculite + Kaolin	CVK	2.90[e]	0.32[j]	1.85[g]
7.5% Cellulose + Talc + Kaolin + Bent	CTKB	4.48[a]	1.59[g]	3.95[b]
7.5% Cellulose + Talc + Kaolin +Verm + Bent	CTKVB	4.25[b]	3.60[c]	3.12[d]

S. Em = 0.168, CD (P = 0.05) = 0.474, *Mean followed by same letters are not significantly different.

cadaver. However no progeny production was observed in non-formulated cadavers. The results revealed that 8-dpi formulated cadavers stored well for a period of seven days only without any adverse effect on nematode reproduction.

The 4-dpi and 8-dpi formulated were better than non-formulated, whereas 4-dpi was better than 8-dpi in overall performance. The formulated cadavers had better ability to store before application over the non-formulated ones.

Effects on infectivity of emerged IJs

No significant differences were found in infectivity of IJs emerging from cadavers (4-dpi) formulated in different carriers after 14 days of storage except SB, which exhibited 60% mortality after 48 h. Twenty one days after storage, a significant reduction in mortality was recorded in SB, AT, ATKB (33%) and CB, GB (66%) 48 h after exposure of IJs to host insect. The infectivity of IJs was not adversely affected in 4-dpi as in 8-dpi formulated cadavers. Infectivity of IJs emerged from formulated cadavers (8-dpi) recorded 100% mortality in all other treatments except CT, STKB, AK (66%) and GT (33%), which exhibited significantly reduced mortality.

Progeny production of *H. indica* 13.3, 6.71 and *H. bacteriophora* formulated 4-dpi cadavers stored for one month in Experiment II

Significant differences were observed in progeny production among carrier materials and not within nematodes species/isolates. Cadavers formulated in CTK, CTKB and CTKVB yielded maximum progeny of *H. indica* and the latter two of *H. bacteriophora*. Progeny production was however hampered in CVB and CVK for all the three nematode isolated (Table 2). In infectivity assay against *G. mellonella*,

absolute mortality was observed with IJs emerging from CTK formulated cadaves for all the three isolates tested. Carrier materials, CTKB and CTKVB did not after the pathogenicity of *H. indica* isolates whereas *H. bacteriophora* was affected. Based on the reproductive potential, infectivity of IJs and storage of cadavers, CTK formulation was found suitable over other carrier materials.

DISCUSSION

The role of carrier materials on the infected cadavers has been examined in terms of emergence and pathogenicity of the IJs at different periods under storage. The dipping and rolling agents used for formulation played a crucial role in keeping the cadavers intact under storage compared to non-formulated. In general, 7.5% Cellulose as dipping agent and combinations of talc and kaolin as coating agents were found to have no adverse effect on the host cadavers, progeny production and infectivity of emerged IJs. Cadavers formulated in these carriers provided complete protein from rupture, shrinkage and sticking together, acted as a barrier in preventing early emergence of IJs than the non formulated cadavers. The tendency for cadavers to rupture was greater in non-formulated with time as the host tissue is digested by the nematodes and symbiotic bacteria (Kaya and Gaugler, 1993).

Formation of 4-dpi cadavers enabled intact storage up to 1 month at ambient temperature whereas non-formulated for 7 days. Progeny production in formulated cadavers was on par with non-formulated at 7 days of storage. Further storage though resulted in decreased production of IJs, the emerged IJs from the host maintained a viable infection and the infectivity of IJs emerging from cadavers over time was not affected. In non-formulated cadavers, storage beyond seven days lead to shrinkage and drying of larval body and thus progeny production was ceased. The cadaver approach has been acknowledged as Ijs emerging from cadavers has highest infectivity rate and dispersal (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999).

The formulations CTKVB, CVB did not negatively affect nematode reproduction and viability but pathogenicity was adversely affected in all the nematode isolates tested. As the storage prolonged, the unfavourable environment inside the host cadaver may have altered the nematode physiology due to inappropriate desiccation and thus resulted in deterioration of pathogenicity. The 4-dpi cadavers were stored for one month whereas 8-dpi and non-formulated cadavers stored only for a period of 7 days. Host insect cadaver may function as a buffer to extreme environmental conditions enabling Ijs to persist longer (Koppenhofer *et al.*, 1995). The progeny production of 8-dpi was affected adversely due to desiccation leading to shrinkage of larval body showing their non-suitability for formulation and storage. In the present study it was observed that cellulose was the only dipping agent, which adhered uniformly to the insect cuticle and retained moisture in the interior. And the coating of TK evenly protected the water content whereas combinations involving bentonite and vermiculite dehyrated the interior layer. Starch with clay as carrier was found to be the best by Shapiro *et al.* (2001) for cadaver formulation. Our studies revealed that starch in combinations contracted microbial infection on storage at different periods and thus

IJs emerged were least. Gelatin failed to smear uniformly to the insect cuticle and resulted in poor adherence of the coating material giving improper structure and least protection to the larval body resulting in reduced progeny. Formulation with agar as dipping agent formed a thick impermeable layer all over the body of the cadaver thus preventing emergence of IJs.

The study reveals the measure of reproductive efficiency at different period after formulation of the host cadavers and the most effective carrier materials for long term storage of the cadavers prior to application. However since the shelf life of nematodes in formulated cadavers has been found to be one month, it requires improvement for practical use in the field. There is a need to test further the formulated cadaver approach under field conditions and compare the efficacy against application of Ijs formulated in different carriers and in aqueous suspension.

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Life table and intrinsic rate of increase of pseudostem weevil *Odoiporus longicollis* Oliv. on popular banana clones of Kerala

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ABSTRACT: Studies were conducted on the life table of the pseudostem weevil *Odoiporus longicollis* Oliv. on popular banana clones, *viz.*, Njalipoovan, Red banana, Robusta, Palayankodan, Poovan, Nendran and Karpooravally. The net reproductive rate varied from 2.18 (Njalipoovan) to 5.89 (Nendran) in a generation time of 5.6 and 6.36 weeks respectively. The innate capacity for increase varied from 0.14 (Njalipoovan) to 0.36 (Poovan). The finite rate of increase was 1.15 in Njalipoovan and 1.43 in Poovan. Based on the age specific survival and natality, the clones Nendran and Poovan were found more susceptible to *O. longicollis* and better suited for the population build up.

KEYWORDS: *Odoiporus longicollis*, life table, banana clones

INTRODUCTION

Pseudostem weevil *Odoiporus longicollis* Oliv. is a destructive pest of banana causing cent per cent yield loss. The occurrence of this pest in Kerala was first reported in 1989 (Visalakshi *et al.*, 1989). Within a decade, the pest has spread to all parts of the state causing severe loss to the banana farmers. The adults cause damage to the plant by making oviposition punctures on the outer sheath and feeding on the pseudostem . The adult females deposit eggs in air chambers of the outer leaf sheath and the developing grubs tunnel towards the core of the pseudostem. They feed on the central portion of the pseudostem by tunneling upwards and downwards. The first visible symptom of attack is the gummy exudation from the oviposition punctures on the pseudostem. Later feeding holes are visible on the pseudostem. The infested plants show reduction in leaf size and yellowing of leaf lamina. This results in the reduction in bunch weight and finger size. Severely infested plants topple by breaking of the pseudostem. In many instances the farmers could diagnose the incidence of the pest only in the late stage of

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infestation when it may be too late to save the crop. The information on the biology and bionomics are vital in evolving strategies for the management of the pest. Life tables provide an important tool in understanding the changes in population of insect pests during different developmental stages throughout their life cycle (Southwood, 1976). An attempt was made to assess the biological potential of *Odoiporus longicollis* by preparing fertility table in different banana clones.

MATERIALS AND METHODS

Biology of the weevil, *O. longicollis* was studied in seven popular banana clones *viz.* Njalipoovan (AB), Red banana (AAA), Robusta (AAA), Palayankodan (AAB), Poovan (AAB), Nendran (AAB) and Karpooravally (ABB). Pseudostem of Nendran was used for rearing the insect with the purpose of maintaining a stock culture. The pseudostem was cut into pieces of 15 cm length and small holes were made at one cut end and grubs collected from the field were introduced in to the holes. These pseudostem with grubs were kept in glass troughs and pseudostem pieces were changed once in two days till pupation. After pupation, pupae were collected and kept for adult emergence. Newly emerged adults were released into fresh pseudostem pieces of each clone kept in a rearing bottle of 20 cm height and 7 cm diameter. The bottles were then closed with nylon net. The eggs laid in the air chambers of the banana sheath were collected daily. Eggs were examined every day for the emergence of grubs. Emerged grubs were separated and fresh pseudostem pieces were given every day till pupation. After pupation, the cocoons were collected and kept in petridishes for adult emergence. Emerged adults were collected and males and females were separated. Observations were recorded. The age specific survival (lx) and age specific fecundity (mx) at each pivotal age (x) were worked out daily for the entire reproductive period. Life tables were constructed for each clone according to the method suggested by Birch (1948); Atwal and Bains (1974).

RESULTS AND DISCUSSION

There was significant difference in the pre-oviposition periods of the insect in different banana clones (Table 1). The pre-oviposition period is longest in Njalipoovan (27.67 days) and shortest in Poovan (13.0 days). A longer preoviposition period indicates a slower rate of development of the reproductive organs which resulted in the delayed physiological maturity. The lowest number of eggs laid by a single female was found in weevils reared in Njalipoovan and Palayankodan (7.07) and the highest number in Karpooravally (13.73). The distance from the epidermis to the air chamber might be an important factor in determining the ovipositional preference of the weevil to a particular clone of banana under field condition (Lalitha *et al.*, 2000). However in a no choice situation under laboratory conditions nutritional factors of the host plant may also found responsible for the variation in the number of eggs laid by an insect. The incubation period ranged from 2.03 to 3.43 days. The longest first instar duration of 5.40 days was noticed in Njalipoovan. Those reared on red banana showed

TABLE 1. Characteristics of *O. longicollis* when reared in popular banana clones

Clones	Pre-oviposition period (days)	Fecundity (number)	Incubation period (days)	I instar (days)	II instar (days)	III instar (days)	IV instar (days)	V instar (days)	Pupal period (days)	Developmental period (days)	Longevity female (days)	Longevity male (days)
Njalipoovan	27.67	7.07	3.43	5.40	3.38	3.29	4.18	7.26	16.18	43.12	128.50	103.14
Red banana	17.00	10.13	2.82	2.38	2.87	3.10	3.32	12.57	13.50	40.69	177.44	151.83
Robusta	25.67	9.60	3.27	2.46	3.61	2.93	4.84	9.54	14.50	41.15	147.92	113.33
Palayankodan	18.67	7.07	2.25	2.60	3.31	4.47	4.22	11.22	13.39	41.46	124.87	87.47
Poovan	13.0	10.47	2.56	3.70	3.88	3.52	4.31	11.89	12.70	42.56	147.25	120.81
Nendran	15.67	12.21	2.60	2.74	2.85	3.62	3.99	10.02	14.58	40.40	143.78	113.19
Karpooravally	18.0	13.73	2.03	2.65	3.47	3.37	5.05	9.20	15.72	41.49	109.81	94.94
CD (0.05%)	2.911	1.823	0.540	0.54	0.57	0.42	0.60	1.42	1.89	3.970	38.580	26.500

TABLE 2. Age specific survival and natality of *Odoiporus longicollis* in popular clones of banana

Pivotal age (weeks)	Njalipoovan		Red banana		Robusta		Palayankodan		Poovan		Nendran		Karpooravally	
	lx	mx	lx	mx	lx	mx	lx	mx	lx	mx	lx	mx	lx	mx
1	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
2	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.076	1.00	0.078	1.00	0.00
3	1.00	0.00	1.00	0.409	1.00	0.223	1.00	1.152	1.00	1.787	1.00	0.824	1.00	0.00
4	1.00	0.034	1.00	0.909	1.00	0.483	1.00	0.576	1.00	1.255	1.00	0.784	1.00	0.308
5	1.00	1.024	1.00	1.455	1.00	0.372	1.00	1.123	1.00	0.076	1.00	0.196	1.00	0.462
6	1.00	0.204	1.00	0.273	1.00	0.223	1.00	0.165	1.00	0.190	1.00	0.235	1.00	0.769
7	1.00	0.614	1.00	0.455	1.00	0.409	1.00	0.041	1.00	0.076	1.00	0.431	1.00	1.846
8	1.00	0.240	1.00	0.545	1.00	0.372	1.00	0.206	1.00	0.00	1.00	0.392	1.00	1.231
9	1.00	0.00	1.00	0.091	1.00	0.149	1.00	0.412	1.00	0.00	1.00	0.863	1.00	0.385
10	1.00	0.240	1.00	0.818	1.00	0.595	1.00	0.288	1.00	0.038	0.95	0.549	1.00	0.192
11	1.00	0.00	1.00	0.136	1.00	0.297	1.00	0.041	1.00	0.076	0.95	0.039	0.85	0.00
12	1.00	0.00	1.00	0.091	1.00	0.00	0.90	0.00	1.00	0.038	0.95	0.510	0.85	0.077
13	1.00	0.034	1.00	0.00	1.00	0.335	0.65	0.041	1.00	0.144	0.95	0.549	0.75	0.038
14	0.80	0.034	0.95	0.00	1.00	0.112	0.25	0.00	0.85	0.038	0.95	0.549	0.60	0.077
15	0.70	0.00	0.85	0.00	1.00	0.037	0.25	0.00	0.80	0.038	0.85	0.00	0.60	0.077
16	0.60	0.00	0.85	0.00	1.00	0.00	0.25	0.00	0.70	0.00	0.75	0.00	0.40	0.00
17	0.60	0.00	0.70	0.00	1.00	0.00	0.20	0.00	0.60	0.00	0.65	0.00	0.25	0.00
18	0.30	0.00	0.65	0.00	1.00	0.00	0.20	0.00	0.50	0.00	0.60	0.00	0.25	0.038
19	0.15	0.00	0.65	0.00	1.00	0.00	0.20	0.00	0.35	0.00	0.50	0.00	0.25	0.00
20	0.15	0.00	0.50	0.00	0.90	0.00	0.15	0.00	0.35	0.00	0.40	0.00	0.15	0.00
21	0.10	0.00	0.50	0.00	0.80	0.00	0.15	0.00	0.30	0.00	0.30	0.00	0.10	0.00
22	0.10	0.00	0.40	0.00	0.60	0.00	0.00	0.00	0.20	0.00	0.15	0.00	0.00	0.00
23	0.10	0.00	0.40	0.00	0.50	0.00			0.20	0.00	0.10	0.00		
24	0.00	0.00	0.30	0.00	0.50	0.00			0.20	0.00	0.05	0.00		
25			0.30	0.00	0.00	0.00			0.10	0.00	0.00	0.00		

Age specific survival (lx). Age specific fecundity (mx).

TABLE 3. Life table statistics of *O. longicollis* in popular banana clones

Clones	Net reproductive rate	Mean generation time (weeks)	Corrected generation time (weeks)	Innate capacity for increase	Finite rate of increase
Njalipoovan	2.180	7.31	5.60	0.14	1.15
Red banana	5.182	6.35	5.59	0.29	1.34
Robusta	3.607	7.95	7.08	0.18	1.19
Palayankodan	3.031	5.51	4.76	0.23	1.26
Poovan	3.790	4.39	3.72	0.36	1.43
Nendran	5.891	8.13	6.36	0.28	1.32
Karpooravally	5.389	7.03	6.87	0.25	1.28

shorter duration (2.38 days). In the clones, Poovan, Robusta and Karpooravally, the second instar grubs recorded duration of 3.88, 3.61 and 3.47 days respectively. The longest duration of third instar grub was recorded in Palayankodan (4.47 days). The shortest duration was observed in grubs from Robusta (2.93 days). In Karpooravally fourth instar grub took 5.05 days whereas Red banana took only 3.32 days. The grubs completed the fifth instar in 12.57 days in Red banana and 7.26 days in Njalipoovan.

Longer pupal period observed was in Njalipoovan (16.18 days) and the shorter period of 12.70 days in Poovan. The total developmental period in Nendran was 40.40 days and in Njalipoovan it was 43.12 days. Weevils reared in Njalipoovan showed longer egg period and pupal period with shorter larval period, indicating the unsuitability of the host plant. The longevity of males as well as female showed significant variation. The longevity of female (177.4 days) and male (151.83 days) was higher when reared in Red banana.

The age specific survival and natality of *O. longicollis* in different banana clones are given in Table 2. In Njalipoovan egg laying started from the fourth week and extended up to fourteenth week. The birth rate was highest (1.024) during fifth week. By the end of eighteenth week, seventy per cent of the weevils died and hundred per cent mortality occurred by the end of twenty third week. In Red banana the weevils oviposited from the third week to the twelfth week of their life . The maximum progeny production (1.455) was recorded in the fifth week. Adult longevity varied from fourteen weeks to thirty weeks. The death of the adult weevil started from the twentieth week onward in Robusta. Egg laying started from the third week and extended up to fifteenth week. In Palayankodan , the weevils oviposited from the third week to thirteenth week. The maximum progeny production of 0.576 was recorded in the fourth week. Hundred per cent mortality of adult occurred by the end of twenty seventh week in Poovan and the birth rate was highest (1.787) during the third week. In Nendran, the highest birth rate was only 0.863 in the ninth week, but the egg laying started from the second week and extended up to fourteenth week. The highest birth rate of 1.846 was recorded in seventeenth week in Karpooravally and egg laying started from the fourth week.

The fertility table of *O. longicollis* in different banana clones categorically gave the picture of susceptibility or resistance of the clones (Table 3) The net reproductive rate varied from 2.18 in Njalipoovan to 5.89 in Nendran in a generation time of 5.60 to 6.36 weeks. The innate capacity for increase which is a statistic parameter of the intrinsic rate of natural increase varied from 0.14 (Njalipoovan) to 0.36 (Poovan). As the intrinsic rate is the infinitesimal rate of increase, the finite rate of increase worked out in the present study varied from 1.15 in Njalipoovan to 1.43 in Poovan. From the table of age specific survival and natality, the clone Nendran and Poovan can be considered as better suited one for the population development of the insect and indicating their susceptibility to *O. longicollis*. Many workers have reported high susceptibility of Nendran to the infestation of *O.longicollis*. Visalakshi *et al.* (1989) reported that the clones Nendran and Red banana were highly susceptible to the weevil attack. Jayasree (1992) reported that the clones Nendran, Poovan and Palayankodan were highly susceptible to *O. longicollis*. The results of the present study support these findings with indepth basis interms of the life table and intrinsic rate of the pest.

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On a new genus and a new species of Entedoninae (Hymenoptera: Chalcidoidea: Eulophidae) from India

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ABSTRACT: A new genus *Bomyiabius* Narendran and a new species *Bomyiabius frontus* Narendran are described from Kerala state (India). The diagnosis and difference with other related genera are also provided.

KEYWORDS: Eulophidae, *Bomyiabius*, new species, India

INTRODUCTION

Eulophidae is one of the economically important families of Chalcidoidea. Many species of this family are parasitic on several species of insect pests of agricultural crops. Unfortunately their taxonomy at alpha level is still incomplete especially in Kerala where rich unexplored fauna awaits discovery. During the course of an inventory on the eulophid fauna of Kerala, we came across an interesting taxon of Entedoninae, which neither fits, to any of the available important keys (Hayat, 1985; Trjapitzyn and Koslyukov, 1987; Boucek, 1988; Mani, 1989 Schauff *et al.*, 1997) nor to any of description of known genera or species of Entedoninae. Hence this interesting genus and species are described below.

The types of new species are deposited in the TCN collections of Systematic Entomology Laboratory, Department of Zoology (DZUC), University of Calicut.

Abbreviations

DZUC = Department of Zoology, University of Calicut; F_1–F_3 = Funicular segments 1 to 3; MV = Marginal vein; OOL = Ocellocular line; PMV = Postmarginal vein; POL = Postocellar line; STV = Stigmal vein; T_1–T_7 = Tergites 1 to 7.

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***Bomyiabius* Narendran gen. nov.**

Type species: *Bomyiabius frontus* Narendran sp. nov.

Diagnosis

Head stout, non collapsing with *X* shaped frontal grooves; occipital carina sharp, postocciput steeply declining; mouth small, narrowed, lower face slightly compressed from sides; In side view mandibles looks swollen, bulging posteriorly; eyes slightly concave on anterior inner orbits on lower side; funicular segments petiolate, clava with apical spicule. Pronotum with anterior margin carinate; mesoscutum with notauli distinct only anteriorly. Propodeum with a median strip of carinate margins, plicae parallel, not converging to nucha, with a pit on either side of each plica on inner basal side, petiole reticulate-granulate, T_1 longest, last tergite about 2.2*x* as long as preceding one

DISCUSSION

Bomyiabius gen.nov. comes in between *Pediobomyia* Girault (Girault, 1913) and *Pediobius* Walker (Walker, 1846). It resembles *Pediobomyia* in having small narrow mouth; lower face narrowly comes in median part and in similar sculpture on scutellum. It differs from *Pediobomyia* in having parallel plicae on propodeum (In *Pediobomyia* plicae converging towards nucha); a pit on either side of each plica basally (not so in *Pediobomyia*); propodeal median strip not granulate (granulate in *Pediobomyia*) and last tergite 2.2*x* as long as preceding tergite (not so in *Pediobomyia*, much shorter).

Bomyiabius resembles *Pediobius* in having similar type of reticulations on dorsum of mesoscutum and scutellum and in parallel plicae on propodeum. However it differs from *Pediobius* in having narrow lower face, smaller mouth (not so in *Pediobius*), parallel plicae of propodeum not widely spaced as those of *Pediobius*. *Bomyiabius frontus* resembles the African *Pediobius acraconae* Kerrich (Kerrich, 1973) in the general shape of gaster but differs from it in generic characters.

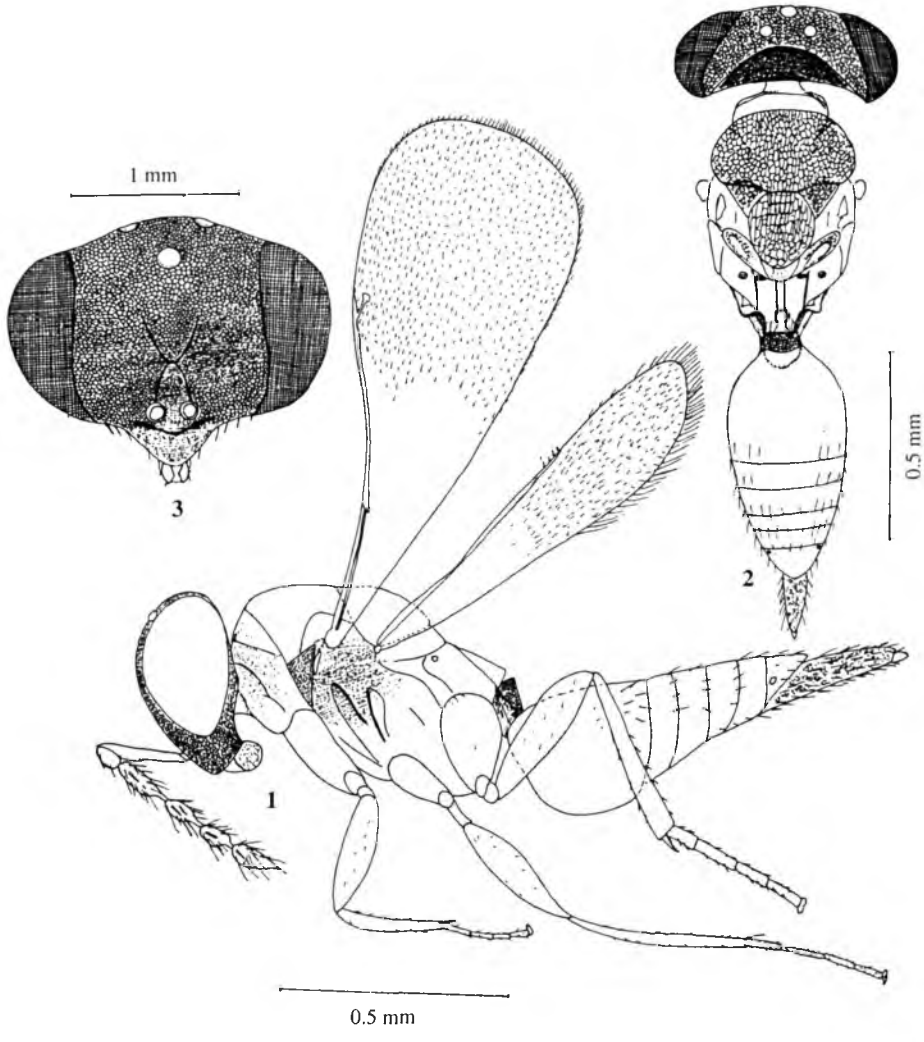
Etymology

The genus name is taken from *Pediobomyia* and *Pediobius*. Masculine gender. The species name after the narrow lower frons.

***Bomyiabius frontus* Narendran sp. nov. (Figs. 1–3)**

Female

Length 1.67–1.69 mm. Black; eye dark brown; ocelli pale reflecting yellow; antenna dark brown with scape pale yellowish brown; median and submedian areas of propodeum and T_1 with metallic greenish blue reflections. Legs pale brownish yellow with fore and mid coxae, fore and hind femur (except bases and apices) darker; basal one third of hind coxa dark with metallic green reflections; pretarsi dark brown; pubescence pale brown except the darker hairs on last tergite.



FIGURES 1–3: *Bomyiabius frontus* Narendran sp.nov. Female 1. Body Profile 2. Body dorsal view 3. Head front view

Head

(Figs 2 and 3) Width 1.29*x* its median length; frons and vertex distinctly reticulate; head width in dorsal view 5.04x its median length (excluding postocciput). POL slightly more than OOL (8 : 7); antennal toruli at level of lower ocular margin; antennal formula 11032. Relative length: width of antennal segments: scape = 22:4; pedicel = 7:4; F1 =16:5; F2 =11:5, F3 =10:5, clava =15:5 with a spicule at apex.

Mesosoma

Pronotum smooth; mesoscutum distinctly and closely reticulate; axilla not well advanced anteriorly, scutellum longitudinally reticulate on two- third area from basal margin, remaining part without in a definite deviation; metanotum with a cup shaped median part. Propodeum (Fig. 2) with submedian area shiny, posterior part with a short nucha. Forewing length 2.47*x* its maximum width, pilose on distal half, proximal half bare; relative length of costal cell 32, MV 51, PMV 5 and STV 5.

Metasoma

(Figs 2 and 3) Petiole 1.75*x* as long as its width in dorsal view; T_1 length 0.3*x* length of gaster in dorsal view; 0.33*x* length of gaster in side view, gaster a little longer than head plus mesosoma; T_4 to apex more pubescent than T_1 to T_3.

Male

Unknown

Host

Unknown

Material examined

Holotype. Female, India: Kerala, Thiruvananthapuram, Mannamkonam, 16.iv.2003. T. C. Narendran and Party. Reg. No. MoEF 2776 (DZUC). Paratypes: 1 Female, India: Kerala, Ernakulam, Kalady, 12.i.2004. T. C Narendran & party Reg. No. MoEF 2050 (DZUC); 2. Female, India: Kerla, Idukki, Vandiperiyar, Pasumala 8.i.2004. T. C Narendran and party. Reg. No. MoEF 1957 (DZUC).

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Article No. ent.29409

The whitefly genus *Martiniella* Jesudasan and David (Aleyrodidae: Hemiptera), with description of one new species from India

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ABSTRACT: A new species of whitefly *Martiniella papillata* infesting on *Xeromphis spinosa* in Goa (India) is described. A key to the Indian species of the genus is given.

KEYWORDS: Indian Aleyrodidae, new species, *Martiniella*

INTRODUCTION

In India, the family Aleyrodidae includes 274 species under 58 genera. Jesudasan and David (1990) proposed the genus *Martiniella* for two species *viz.*, *Aleurotuberculatus canangae* and *Aleurotuberculatus macarange* described by Corbett (1935), using the much elongated, jointed cephalic and first abdominal setae as the diagnostic separation from *Aleuroclava* Singh. Martin (1999) commented that, although unusual, setae of this type are sometimes present in species of *Taiwanaleyrodes* Takahashi and *Dialeurodes* Cockerell and this character seemed to vary within samples (as observed by him)". Therefore, he considered *Martiniella* as junior synonym of *Aleuroclava* Singh. However, we still uphold the validity of the genus *Martiniella* as the jointed setae are always distinct in all specimens studied and there has been no instance of non-jointed setae being observed. In having thoracic tracheal clefts, *Martiniella* is distinct from *Taiwanaleyrodes*. Further, two-segmented setae, observed as a constant characteristic feature in all specimens studied is absent in *Aleuroclava*. Hence, *Martiniella* is retained here as a valid genus. In this paper, the characteristic features of *Martiniella* are redefined with description of a new species.

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Genus *Martiniella* Jesudasan and David

Type species

Aleurotuberculatus canangae Corbett, 1935. *J. Fed. Malay. St. Mus.* **17**: 827–828; by original designation.

Diagnosis: Puparia elliptic with dorsal tubercles and granules; margin crenulate; thoracic tracheal folds and clefts present, caudal fold distinct; cephalic and first abdominal setae always two-segmented. In some species submargin with a row of papillae or thoracic tracheal folds with stipples. Vasiform orifice subcordate, notched at hind end; operculum filling orifice, obscuring lingula.

KEY TO THE INDIAN SPECIES OF *MARTINIELLA*

1. Submargin without a row of papillae; median tubercles absent on abdominal segments 2
– Submargin with a row of papillae; median tubercles present on abdominal segments ..... *papillata* sp. nov.
2. Margin with 30–36 crenulations in 0.1 mm; subdorsum without granules; thoracic tracheal folds with stipples; vasiform orifice cordate ..... *ayyari* Sundararaj and David
– Margin with 29–30 crenulations in 0.1 mm; subdorsum with granules; thoracic tracheal folds without stipples; vasiform orifice subrectangular ..... *lefroyi* Sundararaj and David

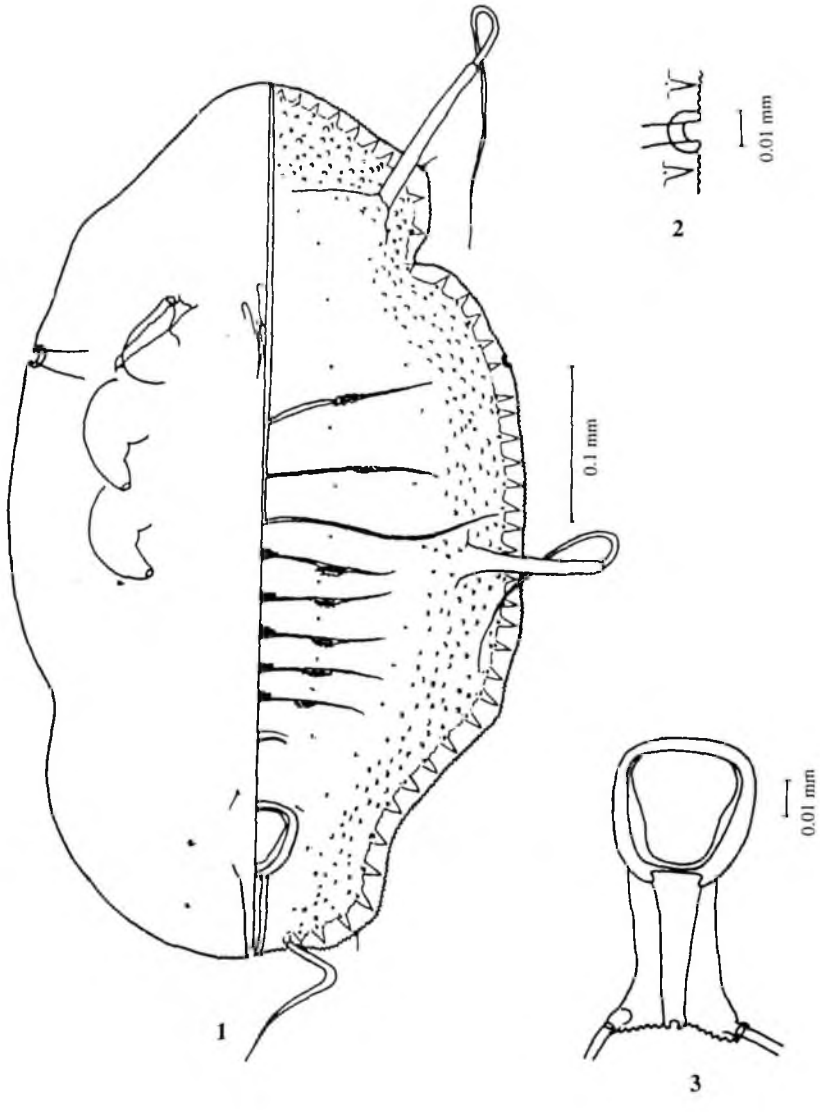
Description

Martiniella papillata sp. nov. (Figs 1–3)

Puparium: Small, elliptical, white, without secretion of wax; broadest at mesothoracic segment, 0.58–0.62 mm long, 0.37–0.54 mm wide; found singly on the under surface of leaves. Margin faintly crenulate, 25–26 crenulations in 0.1 mm. Anterior and posterior marginal setae 6 and 16 μm long respectively. Thoracic and caudal tracheal pores distinct.

Dorsum: Submargin with a row of papillae each with a minute pore at base; median tubercles on abdominal segments I–V distinct, subdorsum with minute tubercles. Longitudinal moulting suture reaching margin and transverse moulting suture reaching submargin. Median length of abdominal segment VII shorter than VIII.

Chaetotaxy: Two pairs of two-segmented setae- cephalic setae 354 μm long, first abdominal setae 364 μm long; a pair of minute eighth abdominal setae cephalolaterad of vasiform ortifice 9 μm long and a pair of submarginal caudal setae 150 μm long.



FIGURES 1–3: *Martiniella papillata* Sundararaj and Dubey, sp. nov. 1. Puparium; 2. Thoracic tracheal pore; 3. Vasiform orifice.

Vasiform orifice: Cordate, notched at hind end, 51–55 μm long, 42–54 μm wide; operculum subcordate, 24–34 μm long, 28–36 μm wide. Lingula tip spinose and included. Thoracic tracheal furrows not indicated while caudal tracheal furrow funnel shaped, 36–52 μm long, 19 μm wide at its widest end. Pores and porettes evident.

Venter: A pair of ventral abdominal setae 14 μm long, 24–34 μm apart. Antennae reaching base of prothoracic legs.

Materials examined

Holotype puparium, India: Goa, Volpoi, one puparium on slide *Xeromphis spinosa*, 27.ii.2001, A. K. Dubey, deposited in Forest Entomology Division, Forest Research Institute, Dehra Dun, India, Paratype, one puparium on slide with data as of holotype, deposited in the collection of the Natural History Museum, London, United Kingdom.

Entomology

Named to reflect its submarginal row of papillae.

Comments

This species resembles *Martiniella canangae* (Corbett) in the presence of submarginal papillae and notched vasiform orifice, but differs in having distinct median tubercles and absence of submedian tubercles on cephalothorax and segment sutures.

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Effect of plantation programmes on insect species diversity — a study in the Parambikulam forest, Kerala, India

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ABSTRACT: The effect of teak monoculture on forest insect diversity was studied in representative plots at Parambikulam Wildlife Sanctuary (Kerala, India). The plots were laid out in such a way that both plantation as well as natural forests at various levels of vegetation diversity was represented. The study revealed that the floral and faunal elements of each of these habitats showed specialization. While the natural forests had good representation of primary tree species like *Palaquium ellipticum*, *Aglaia* sp., *Myristica dactyloides*, *Mesua ferrea*, *Cullenia exarillata*, *Holigarna arnottiana*, *Casearia bourdiloni* and *Persea macrantha*, the plantations had poor representation of these species and showed invasion of secondary tree species like *Scolopia crenulata*, *Walsura trifolia*, *Celtis* sp., *Albizia chinensis*, *Macaranga indica* and *M. peltata* and weeds like *Clerodendrum viscosum*, *Mikania micrantha* and *Lantana camara*. The faunal composition in the two areas was also found to be distinct. While the undisturbed areas contained more of arboreal feeding insects belonging to the families, Geometridae, Saturnidae and Cossidae, the disturbed areas were abundant in herbaceous feeding forms belonging to the families, Pyralidae, Noctuidae and Chrysomelidae. The diversity indices for plants and insects calculated separately for each of the habitats also showed marked difference with the area under teak monoculture showing a reduction in species diversity both of flora and fauna. There was also difference in the values of similarity index calculated for these habitats. The insect and plant diversity indices were also significantly correlated, suggesting the adverse effect of forest disturbance on insect species diversity.

KEYWORDS: Forest insect diversity, effect of plantation activities, Parambikulam Wildlife Sanctuary

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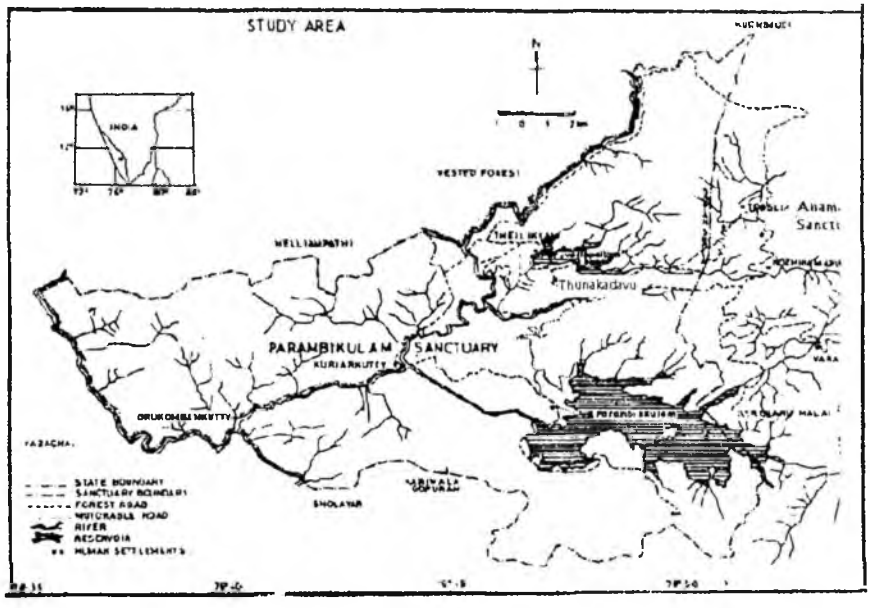

FIGURE 1. Study Area

INTRODUCTION

The importance of biodiversity for maintaining life support systems of the biosphere is well recognized. An account of studies carried out on various insect groups from the Kerala part of Western Ghats is given by Mathew (2004). These studies have shown that the Kerala part of W. Ghats is extremely rich in species diversity and several locations are considered biodiversity 'hotspots'. However, large areas in this region are under considerable anthropogenic pressure and many species are likely to become extinct if appropriate conservation strategies are not undertaken. Incidence of fire, plantation programmes, cutting of trees for fodder and firewood and cattle grazing are the important disturbances in these areas. Documentation of biodiversity and evaluation of the impact of various disturbances on species diversity patterns are fundamental to any conservation programme. In this study, an attempt was made to assess the impact of forest plantation programmes on insect species diversity in the Parambikulam Wildlife Sanctuary.

MATERIALS AND METHODS

Study area

The Parambikulam Wildlife Sanctuary (Fig. 1) covering an area of 270 km^2 is located in the Palghat district of Kerala State. The Sanctuary, which lies between 76°35′ and 76°50′E and 10°20′ and 10°26′N at an elevation of 600 m above sea level

(Viswanathan, 1958; Uniyal, 1987) came into existence in 1962 by setting apart the Sungam Range of Nemmara Forest Division to which the Parambikulam Range was added in 1973. The boundaries of this Sanctuary include the Indira Gandhi Wildlife Sanctuary of Tamil Nadu on the east, the Nelliyampathy Reserve Forests on the north-west and the Vazhachal and Sholayar forests on the south.

The terrain is more or less hilly with undulating plains interspersed with marshy fields in the valleys. The altitude varies between 600 and 1400 m, and the highest peak is the Karimala Gopuram, which has an elevation of 1438.96 m. The mountain slopes are non-symmetrical and non-uniform, spread throughout the area in different directions. The ridges of the Sanctuary are of sheet rock and are exposed at the top. Some of the hilltops have a thin crust of soil favoring stretches of grasslands.

The climate in general is pleasant. The maximum temperature ranges between 24 °C to 33 °C and minimum, 20 °C to 25 °C. The average annual precipitation is 1800 mm varying between 1200 to 2300 mm. The area gets both the southwest and northeast monsoons, the southwest monsoon being more active. When compared to the eastern portion, the western part of the Sanctuary receives higher precipitation. The monsoons last from June to December and as such, two seasons i.e., dry and wet could be distinguished in the area. The months January to May with low or no rainfall is considered to be the dry season.

Forest types

A wide variety of habitats, both natural and man-made are available within the Sanctuary. Based on the classification by (Champian and Seth, 1968), the natural vegetation in this area can be classified broadly as West-Coast tropical evergreen (55 km^2), and the South Indian dry deciduous (15 km^2) forests. Semi-evergreen forests appear where moist deciduous forests merge into evergreen forests. Grasslands are seen above 1000 m on the upper reaches of Karimala Gopuram and Vengoli hills. Small patches of bamboo and reed are also present in certain areas. The man-made habitats are chiefly teak plantations covering an area of about 90 km^2; the first plantations were raised in the year 1912. In addition to this, a small area of the Sanctuary along the Tamil Nadu border is planted with eucalyptus.

The raising of teak plantations after clear felling of natural forests is the major disturbance to the forest biota in the Parambikulam Wildlife Sanctuary. However, being a Wildlife Santuary, the plantations are left without much weeding or extraction of miscellaneous tree species because of which some areas have developed into mixed stands.

Sampling methods

The study was carried out in representative plots taken along a transect in such a way that both disturbed (teak plantation) and undisturbed areas were covered. The teak plantations were over 30 years old having good underground vegetation. Altogether 8 plots were taken, of which four plots were in the disturbed and four in the undisturbed areas. The plot size was 625 m^2 (25 m × 25 m) and the distance between plots was

25 m. Data on vegetation and insects were collected from all the eight plots in each locality and from this, indices of diversity, dominance, evenness and species richness of plants and insects were computed separately for the disturbed and undisturbed plots. The values for the disturbed and undisturbed areas were pooled for deriving the overall values for each locality. Details of methodology followed for studies on vegetation and insect community are described below.

Vegetation studies

Vegetation was studied with a view to generate base-line data on the floral elements to facilitate comparison of the relationship between the vegetation and insect community. For this, plants above 2 cm in diameter were enumerated in all the study plots. The diameter of small plants was measured at about 6 cm from ground. In the case of tall plants, girth at breast-height (gbh) was recorded. Based on this, the tall plants were classified into different categories *viz.*, mature trees (individuals with gbh more than 30.1 cm), saplings (individuals with gbh 10.1 to 30 cm), seedlings, shrubs, herbs and climbers (individuals with girth < 10 cm) (Chandrashekara and Ramakrishnan, 1994).

Insect community studies

Sampling of insects was done using a battery operated light trap, specially fitted with a switching device to facilitate self-operation at specified hours (Mathew and Rehamathulla, 1995). The trap was fitted with solar panels to facilitate charging of battery during the day. In order to avoid the influence of lunar phase on insect catches, the trap was operated alternately between plots in the disturbed and undisturbed areas i.e., if the trap was operated initially in plot 1 in the disturbed area, on the next day, it was operated in the plot 1 of the undisturbed area and then in plot 2 of the disturbed area and so on. In addition to trap catches, collections were also made during daytimes (8 am to 1 pm) using hand nets. Collections were made for a period of one year and the insects collected were sorted out to species and the number of individuals for each species was recorded. As it was not possible to identify all the species readily, code numbers were assigned to the various species. The insects were later identified by comparison with material available in the national collections at IARI, New Delhi and ZSI, Calcutta, by the first author and by referring to experts in these institutions.

Analysis of data

Density

Density was estimated for various plant categories such as trees, saplings, seedlings, shrubs, herbs and climbers in each locality for disturbed and undisturbed sites and the pooled values were also calculated separately.

Diversity index

Shannon-Weiner diversity index was calculated as given in Margalef (1968). In order to find out whether any significant difference existed in the insect diversity of the disturbed and undisturbed areas, a '*t*' test was done (Magurran, 1988).

Distribution models

This is another way of describing diversity in a community (Fisher *et al.*, 1943). A species-abundance model utilises all the information gathered in a community and is the most complete mathematical description of the data (Magurran, 1988). The frequency distribution of insects per collected species was studied and the data were described using truncated log-normal distribution (Pielou, 1975), which will indicate whether the locality contain any rare species or not and also, the number of species which had not been possibly included in the sample collection.

Similarity measures

The similarity between disturbed and undisturbed areas was studied by calculating the Sorensen's coefficient of similarity (modified by Bray and Curtis (1957)).

Dominance index

The dominance index was calculated for studying the patterns of relative abundance of each insect Order in the study site.

Evenness or equitability index

This index, which measures the evenness of species abundance, is complementary to the diversity index concept and it indicates how the individuals of various species are distributed in the community. For estimating evenness, Shannon's evenness index was calculated (Pielou, 1975, 1977).

Species richness

In the ecological literature, the number of species at a site, in a region or in a collection is called species richness, which is the simplest and most useful measure of species diversity. In this study, the total number of insect species collected from all the plots separately from each locality was considered as species richness.

Species richness index

It would appear that an unambiguous and straightforward index of species richness would be the total number of species in a community *S*. However, since *S* depends on the sample size (and the time spent for searching), it is limited as a comparative index (Yapp, 1979). Hence, a number of indices have been proposed, and Menhinick's index (1964) which is widely applied (Ludwig *et al.*, 1988) was used in this study.

RESULTS

Vegetation studies

Out of 30 plant species recorded in the undisturbed area (natural forest), 21 were represented as mature trees, 11 as tree saplings and 14 as tree seedlings (Table 1). *Aglaia lawii, Cullenia exarillata, Dimocarpus longan, Drypetes elata, D. oblongifolia, Elaeocarpus serratus, Gomphandra coriacea, Haldina cordifolia, Heritera papilio, Holigarna arnottiana, Litsea floribunda, Mastixia arborea, Mesua ferrea, Myristica dactyloides, Neolitsea* sp., *Palaquium ellipticum* and *Polyalthia coffeoides* were the common species. In addition to these, secondary species like *Agrostistachyus meeboldii, Scolopia crenata* and *Laportea crenulata* were present, indicating slight disturbance. Shrubs, herbs, herbs and climbers were sparse.

Density was high for tree seedlings. The species diversity index was highest for mature trees (2.36), followed by seedlings (2.10) and saplings (1.71). The species richness index was also highest for mature trees (2.13) followed by tree seedlings (1.24) and tree saplings (1.07). Evenness index was highest for tree seedlings (0.80), followed by mature trees (0.78) and shrubs (0.78).

In the disturbed area (teak plantation), only 19 species were recorded, of which 5 were represented as mature trees, 9 as tree seedlings and 8 as tree saplings (Table 1). Besides large trees of *Heritiera papilio, Holigarna* sp., *Mastixia arborea, Mesua ferrea* and *Neolitsea* sp., secondary species like *Artocarpus heterophyllus, Alstonia scholaris, Celtis* sp., *Erythrina* sp., and *Macaranga indica* were also present. Shrubs and herbs were sparse and no climber was present. Highest diversity was noticed for shrubs (2.10). Density was low for all categories. Species richness index was higher for tree saplings (1.49). Evenness index was highest for tree saplings (0.74) and nil for herbs and climbers.

At Parambikulam, the disturbed areas showed marked reduction in the number of species which, as expected, was the aftermath of teak monoculture. The small number of miscellaneous species present in the plantations originated through natural regeneration from the adjacent moist deciduous forest patches. The natural forests contained more number of species (Table 1).

Insect community studies

Data on the number of species collected from the disturbed and undisturbed areas at Parambikulam are given in Table 2. Three hundred and fifty one species were recorded from the undisturbed and 292 species from the disturbed areas. Species collected from the undisturbed area belonged to 11 Orders and 62 families and those from the disturbed area, to 9 Orders and 56 families. The identity of 228 species could be established; a list is available with the first author.

Species richness

In the undisturbed area, maximum number of insects was collected in March 1996 (118) followed by February 1996 (115) and least in October 1998 (24). In the disturbed

TABLE 1. Characteristics of the vegetation in the undisturbed and distributed sites at Parambikulam

Community parameters	Category	Plant categories						Total
		Mature trees	Tree saplings	Tree seedlings	Shrubs	Herbs	Climbers	
No. of species	UD	21	11	14	3	3	1	30
	D	15	8	9	6	1	0	19
No. of plants	UD	97	118	127	80	27	3	452
	D	32	29	62	30	18	0	171
Diversity index	UD	2.36	1.71	2.10	0.86	0.42	0	2.68
	D	1.05	1.54	1.14	2.10	0.00	0	2.24
Richness Index	UD	2.13	1.07	1.24	0.34	0.58	0.58	1.41
	D	0.88	1.49	1.14	1.10	0.24	0	1.45
Evenness index	UD	0.78	0.71	0.80	0.78	0.38	0	0.79
	D	0.65	0.74	0.52	1.17	0	0	0.76

UD – Undisturbed; D – Distributed; Plot size: 25 m x 25 m; replicates: 4 plots per habitat.

TABLE 2. Number of species collected at Parambikulam in different months

	1995	1996										Total
Category	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
Undisturbed area	52	67	115	118	71	67	77	75	63	41	24	32	351
Disturbed area	47	53	92	57	95	80	95	51	51	31	30	25	292

area, the number of species collected was higher in April and June 1996, and least in November 1996.

Distinct seasonal changes in species composition were observed during the study period in both the undisturbed as well as disturbed areas. There was also a reduction in the number of species during the monsoon months (July–November). During the summer months (January–May), the number of species collected was relatively high. There was significant difference in insect species richness in various months from the undisturbed $(\chi^2 = 144.45)$ and disturbed $(\chi^2 = 122.45)$ areas.

Species richness index

Species richness index showed high values for undisturbed area (5.62) compared to the disturbed area (4.46) indicating that the undisturbed areas are more diverse.

Dominance index

The dominance indices for various insect groups at Parambikulam are given in Table 3.

TABLE 3. Dominance index of insect groups at Parambikulam

Group	Dominance indices	
	Undistrubed area	Disturbed area
Coleoptera	50.15	60.03
Dictyoptera	0.67	0.91
Diptera	14.16	9.44
Ephemeroptera	0.23	—
Hemiptera	12.17	7.08
Hymenoptera	8.04	17.02
Isoptera	1.97	0.61
Lepidoptera	11.37	4.55
Odonata	0.05	—
Orthoptera	0.74	0.16
Trichoptera	0.44	0.19

TABLE 4. Number of insects recorded in each month at Parambikulam

Area	1995	1996										Total
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
Undisturbed area	358	473	488	681	397	389	303	247	344	93	64	67	3904
Disturbed area	156	255	550	325	1308	555	511	188	262	85	41	52	4288

The dominant insect groups in the undisturbed area were Coleoptera (50.15), Diptera (14.16) and Hemiptera (12.17) while in the disturbed area, they were Coleoptera (60.03), Hymenoptera (17.02) and Diptera (9.44).

Species abundance

The number of insects collected ranged from 64 (October 1996) to 681 (March 1996) in the undisturbed area and from 41 (October 1996) to 1308 (April 1996) in the disturbed area. Least number of insect collected was from September to November 1996 in both the areas (Table 4). The chi-square test showed highly significant difference in the number of insects in various months in undisturbed $(\chi^2 = 1171.16)$ and disturbed $(\chi^2 = 3808.37)$ areas.

Species diversity

Shannon's index of diversity was calculated month wise for various study sites as presented in table 5. In the undisturbed area, the highest diversity obtained was 4.00 (February 1995) and the least 2.59 (December 1995). For the disturbed area, the highest diversity obtained was 3.46 (December, 1995) and the least 2.48 (August, 1996). There was significant difference in the insect diversity between undisturbed

TABLE 5. Species diversity indices for insects collected from Parambikulam

Area	1995			1996									Total
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
Undisturbed area	2.59	2.91	4.00	3.53	3.56	3.61	3.73	3.60	3.20	3.52	2.89	3.31	4.64
Disturbed area	3.46	3.36	3.13	2.80	2.54	2.86	2.93	3.10	2.48	2.84	3.32	2.99	3.99

TABLE 6. Truncated lognormal distribution at Parambikulam

Class	Upper class	Observed	Expected	χ^2
Behind Veil line	0.5	—	52.82	—
1	2.5	144	137.94	0.26
2	4.5	66	66.20	0.00
3	8.5	73	68.59	0.28
4	16.5	50	58.86	1.33
5	32.5	45	41.87	0.23
6	64.5	17	24.81	2.45
7	128.5	13	12.22	0.04
8	256.5	3	5.07	0.84
9	512.5	5	1.77	5.89
10	1024.5	2	0.67	2.64
Total		418	470.82	13.96

and disturbed areas ($t = 11.98$); the undisturbed areas were more diverse than the disturbed areas.

Distribution model

A truncated lognormal distribution was fitted to the data (pooled data from undisturbed and disturbed areas) from Parambikulam. The χ^2 goodness of fit test showed no significant difference between the observed and expected distribution (Table 6). This implies that the distribution pattern of species is following truncated lognormal distribution at Parambikulam $(\chi^2 = 2.993)$. There were 53 species that were not covered in the survey.

Evenness of equitability index

Shannon's evenness index was 0.79 for the undisturbed area and 0.70 for the disturbed area, indicating rich species diversity in the latter.

TABLE 7. Comparison of plant and insect diversity at Parambikulam

Study area	Plant diversity	Plant species	Plant number	Insect diversity	Insect species	Insect number
Overall	2.67	35	623	4.50	418	8192
Undisturbed area	2.68	30	452	4.64	351	4288
Disturbed area	2.24	19	171	3.99	292	3904

Similarity measures

Modified Sorenson's similarity index obtained was 0.48. This shows that there is 48% similarity between disturbed and undisturbed areas at Parambikulam. This is in support of the findings that there is significant difference in species diversity between disturbed and undisturbed areas

DISCUSSION

The study area at Parambikulam contained 623 plants belonging to 35 species and 8192 insects belonging to 418 species. The overall diversity index was 2.67 for plants and 4.50 for insects. The disturbed area had less number of plants and insects compared to the undisturbed area (Table 7).

The number of plants recorded from the disturbed area was 171 belonging to 19 species. With regard to insects, 3904 individuals belonging to 292 species have been recorded. The undisturbed area contained 452 plants belonging to 30 species and 4288 insects belonging to 351 species. Similarly, in the disturbed area, the diversity index was only 2.24 for plants and 3.99 for insects. The values were 2.68 for plants and 4.64 for insects in the undisturbed area. The insect and plant diversity indices were found to be significantly correlated ($r = 73.49\%$ at $P = 0.05$) which suggests that reduction in plant diversity will adversely affect insect diversity of the area.

The results of this study are indicative of the negative impact of forest plantation activities on insect species diversity. Insects being largely dependant on the flora, it is the floral composition that determines the insect species composition of an area. While large scale forest plantation programmes will cause drastic ecological changes leading to habitat loss for many animal communities threatening their survival, subsequent recolonisation of plant communities may offer future survival chances for many animal communities. For instance, the vegetation in the undisturbed areas contained a high proportion of mature trees, tree seedlings and saplings while the disturbed areas contained lesser number of these elements and there was an increase in the number of shrubs and herbs. The undisturbed areas also had good representation of primary species like *Palaquium ellipticum, Aglaia* sp., *Myristica dactyloides, Mesua ferrea, Cullenia exarillata, Holigarna arnottiana, Casearia bourdiloni* and *Persea macrantha*

while in the disturbed areas, only a low proportion of the primary species were present and there was invasion of various secondary species like *Scolopia crenulata, Walsura trifolia, Celtis* sp., *Albizia chinensis, Macaranga indica* and *M. peltata* and weeds like *Clerodendrum viscosum, Mikania micrantha* and *Lantana camara*. Similarly, with regard to insects, the undisturbed area contained more of arboreal feeding insects belonging to the families, Geometridae, Saturnidae, Cossidae etc., whereas the disturbed areas were abundant in herbaceous feeding forms belonging to the families, Pyralidae, Noctuidae, Chrysomelidae etc. Very characteristic biota including rare and endemic species of both plants and insects is almost vanished from the disturbed sites because of their specialised behavioral adaptation for specific habitats/eco-climatic conditions.

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Characterization and cDNA cloning of apolipophorin III gene in the red cotton bug, *Dysdercus cingulatus*

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ABSTRACT: Lipophorin, a major plasma lipoprotein acts as a true carrier of lipids, phospholipids, carotenoids, cholesterol, fatty acid and hormones. It is composed of three distinct apoproteins: apolipophorin-I, apolipophorin-II and a smaller apolipophorin-III. Nucleotide sequencing of apolipophorin-III gene of *D. cingulatus* consists of 615 nucleotides. Amino acid composition showed a high percentage of isoleucine and low percentage of histidine, tryptophan and methionine. Phylogenetic tree of *D. cingulatus* revealed close relationship with the coleopteran insect, *Derobrachus geminatus* while distantly related to lepidopteran insects.

KEYWORDS: *Dysdercus cingulatus*, apolipophorin-III, cDNA cloning

INTRODUCTION

Lipophorin is a dynamic particle that acts as a reusable shuttle, loading lipids at the sites of absorption or biosynthesis and unloading them at storage organs or peripheral tissues. Apolipophorin-III that normally exists as a water soluble monomer haemolymph protein. Diacylglycerol is mobilized from the triacylglycerol stores in the fat body and released into the haemolymph. Mobilization of diacylglycerol is initiated by adipokinetic hormone. These mobilized diacylglycerol are loaded on to the haemolymph high density lipophorin with the help of apolipophorin-III. The full length cDNA sequence of apoLp-III gene from a few of the insect species are already

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known (Cole *et al.*, 1987; Kanost *et al.*, 1988; Van Heusden and Law, 1989; Smith *et al.*, 1994; Yamauchi *et al.*, 1996, 1997; Regland. *et al.*, 1997; Kim *et al.*, 1998; Niere *et al.*, 1999; Liu *et al.*, 2000), but such information in bugs are lacking.

We report here the nucleotide sequence and putative amino acid sequence of apolipophorin-III gene. In addition, detailed data on comparative sequence analysis, amino acid analysis multiple sequence alignment and phylogenetic tree are also presented.

MATERIALS AND METHODS

Experimental animal

The red cotton bug, *Dysdercus cingulatus* was reared in the laboratory on soaked cotton seeds under controlled conditions (27 ± 2 °C; r.h. 90 ± 3 and 12 : 12 L/D cycle). Eggs when hatched were transferred to plastic rearing basins. Newly emerged adults were isolated each morning and were designated as 0-day old. This ensured availability of insects of known age group whenever required. Adult females were used for the present studies.

Isolation of mRNA and cDNA synthesis

Total RNA was extracted from fat body following single step Guanidium thiocyanate method (Chomczynski and Sacchi, 1987). RNA was checked by agarose gel electrophoresis. A modified protocol was used to amplify the 3′- end of the lipophorin cDNA. The reaction mix was incubated at 37 °C for 1 hr to allow the synthesis of the first strand cDNA.

Oligonucleotide primers and RT-PCR amplification of cDNA

Several published apolipophorin-III cDNA sequences of different insects were analysed. Based on their homology in the 3-region, four primers were designed:

F1 : 5′ – GCCGCCAATGCCCAACAGAT-3′
F2 : 5′ – TCGCWCWRCRMRATGKTKCK-3′
R1 : 5′ – CAGAAACGGCATCAAATTAT-3′
R2 : 5′ – TTGAKYKCTTRGCTTSCTT-3′

where M = A/C, R = A/C, W = A/T, Y = C/T, S = C/G, K = G/T.

The first strand cDNA was amplified with F2 and poly T primer using Perkin Elmer Cycler 2400. RT-PCR reactions were carried out according to Sambrook *et al.* (1989) and the resulting products were checked by 1.5% agarose gel electrophoresis.

DNA sequencing and analysis

All the sequences were carried out with an automated DNA sequencer [PE Applied Biosynthesis, ABI Prism 377] using Big Dye terminator Kit [Perkin Elmer, USA]. Nucleotides and translated amino acid sequences were compared to other related

TABLE 1. Pairwise nucleotide similarity of *Dysdercus cingulatus* apoLp - III gene with apoLp - III gene of other insects

Sl. No.	Name of Insect	Accession No.	Nucleotide %	BP Length	Reference
1.	*Epiphyas postvittana*	AF314181	32.0	709	Liu *et al.*, 2000
2.	*Galleria mellonella*	AJ006975	56.0	699	Niere *et al.*, 1999
3.	*Agrius convolvuli*	AF001632	56.0	537	Regland. *et al.*, 1997
4.	*Spodoptera litura*	AF094582	54.0	704	Kim *et al.*, 1998
5.	*Bombyx mori*	U59244	63.9	623	Yamauchi *et al.*, 1996
6.	*Bombyx mandarina*	AF001289	63.9	623	Yamauchi *et al.*, 1997
7.	*Acheta domesticus*	L25277	64.0	882	Smith *et al.*, 1994
8.	*Derobrachus geminatus*	L25278	74.0	659	Smith *et al.*, 1994
9.	*Manduca sexta*	M17286	54.0	708	Cole *et al.*, 1987
10.	*Locusta migratoria*	JO3888	54.0	672	Kanost *et al.*, 1988
11.	*Dysdercus cingulatus*	AF464872	—	**615**	Present study

sequences using BLAST search of the non-redundant sequence database (Altschul *et al.*, 1990). The percentage identity between sequence pairs was calculated using GAP program of GCG package (Devereux *et al.*, 1984, Genetic Computer Group). Multiple sequences were aligned using PILEUP (Devereux *et al.*, 1984, GCG) with gap weight 3.0, gap length weight 0.1. Gen Doc software was used for similarity and scoring among the aligned sequences.

RESULTS

Sequencing of apolipophorin-III gene

Agarose gel electrophoresis of isolated RNA (A), amplified RT-PCR product of mRNA (B), Restriction digestion of clones (C) and Identification of clones by amplification (D) are shown in Fig. 1.

Apolipophorin-III gene of *Dysdercus cingulatus* contained 615 nucleotides as shown in Fig. 2. Percentage similarity in nucleotide sequence of *Dysdercus cingulatus* apolipophorin-III gene was compared with the available ten sequence. Coding sequence of apolipophorin of *Dysdercus cingulatus* is closely related to *Derobrachus geminatus* (74%) while it is distantly related to *Epiphyas postvittana* (32%). Various insects with its, Bp length and Accession No. are represented in Table 1. Composition of amino acid (mol%) of apoLp-III of the bug is shown in Table 2. Phylogenetic tree of *Dysdercus cingulatus* based on apolipophorin-III gene seqquence in comparison to other insects are shown in Fig. 3. This shows a close relationship with *Derobrachus geminatus* and distantly related to lepidopteran insects like *Bombyx mori*, *Bombyx mandarina* and *Galleria mellonella*. Multiple sequence alignment of *Dysdercus cingulatus* apolipophorin-III nucleotide sequence with that of other insects are shown in Fig. 4.

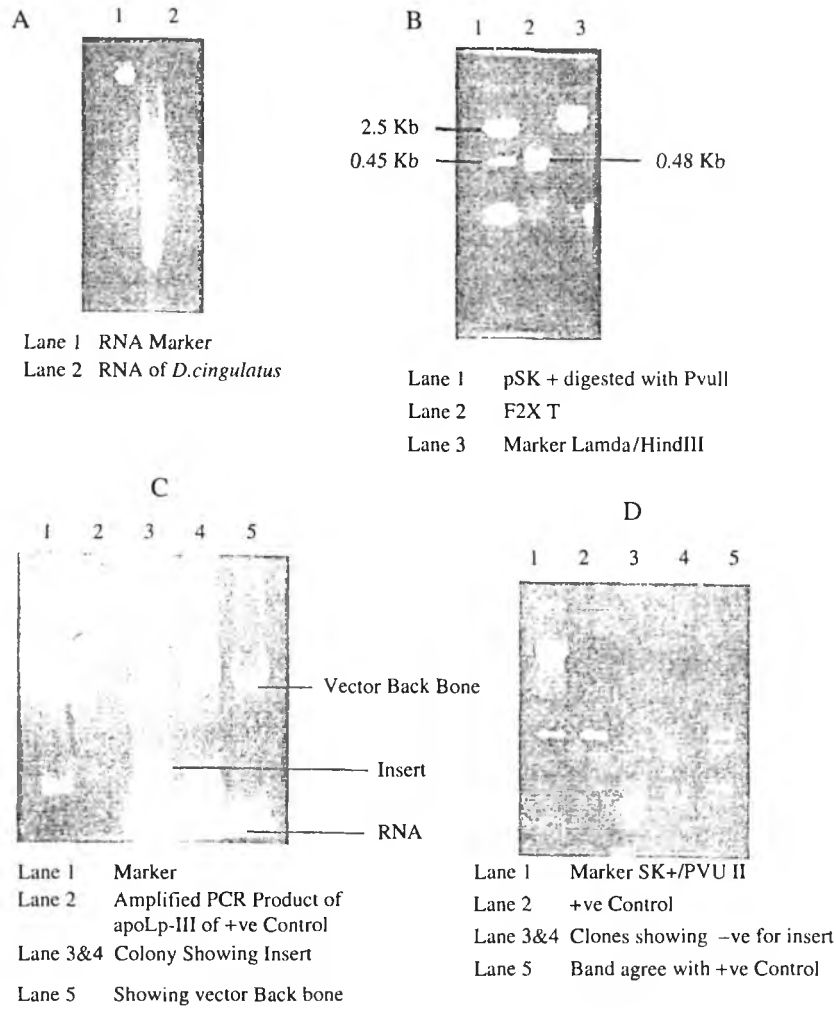


FIGURE 1. Agarose gel electrophorograms showing cDNA amplification.

The nucleotide sequence data of the present study has been submitted to the Gen Bank database and assigned with accession number **AF 464872**.

DISCUSSION

Analysis of the nucleotide sequence revealed that the apoLp-III gene of *D. cingulatus* consisted of 615 nucleotides. It is in close agreement with the published Gene Bank data on nucleotide sequences of other known insect species. Almost similar nucleotide seqeunce for apoLp-III has been reported in *Bombyx mori* (AC No. U 59244) 623

gctgcatgcctgcagctcgactctagaggatctactagtcatatggattcgctcttcgcgatggttcta
acagtggccaagtacattgtaatcgttgttgttgccttgttcgctctacaaatgtttctgctaaaccaag
agccaaagtcgccgaaaatctcaactgcaagaacttgccgccaatgcccaacctaattttgaaaac
aataacaataattttatattaaaacaatgtcgtttattcaattttatcaagcaaactgagcgagaccgca
gctgaattgcaaaaacagttgggaccttggcataacattcattaaggaaaaatcatgtgatttttacct
ataatcataattaacatcaaatgagggccgtaaatatttattaacgataatatatagtacatataacttg
aagaccaaattgtagaagttagtaacaacgtgcaacaacaagtaagaagagctatagttcgaaaaa
ttactaataatttaatttgtttttactatatttgatttttttatttcattttgcttctttatttttgattagataaagttg
gaataattgttttctccaaataaactattattccaagttttgaaaccggtcaa

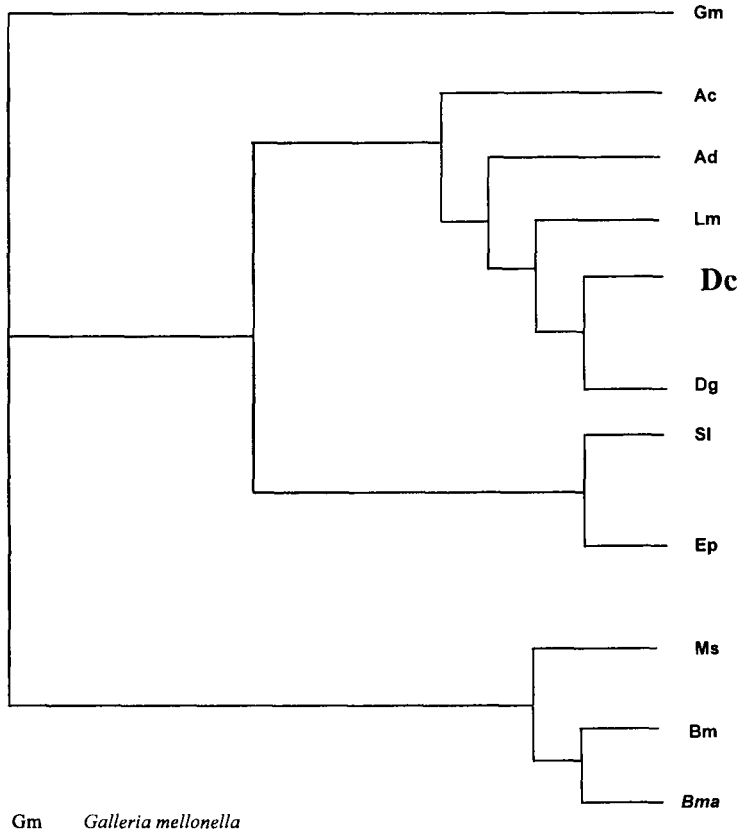
FIGURE 2. Nucleotide sequence of apolipophorin-III cDNA of *Dysdercus cingulatus*.

TABLE 2. Composition of amino acid analysis (mol%) of apoLp-III of *D. cingulatus*

Amino acid	3 let Abbre	1 Let Abbre	Codon	Mol%	Functional	Hydrophobic	Charge	Chemical
Alanine	Ala	A	GCA	4.54	HNP	Pho	0	Aliphatic
Argenine	Arg	R	AGA	3.03	+	Phi	+	Basic
Asparagine	Asn	N	AAC	9.85	POU	Phi	0	Amide
Aspartic acid	Asp	D	GAC	2.27	–	Phi	–	Acidic
Glutamic acid	Glu	E	GAA	2.27	–	Phi	–	Acidic
Glutamine	Gln	Q	CAA	4.54	pou	pHI	0	Amide
Glycine	Gly	G	GGA	2.27	POU	Phi	0	Aliphatic
Histidine	His	H	CAC	1.51	+	Phi	+	Basic
Isoleucine	Ile	I	AUA	15.15	hnp	pHO	0	Aliphatic
Leucine	Leu	L	CUA	7.58	HNP	Pho	0	Aliphatic
Lysine	Lys	K	AAA	8.33	+	Phi	+	Basic
Methionine	Met	M	AUG	1.51	HNP	Pho	0	Sulfur
Phenylalanine	Phe	F	UUC	9.09	HNP	Pho	0	Aromatic
Proline	Pro	P	CCU	6.06	HNP	Pho	0	Imino
Serine	Ser	S	AGC	8.33	POU	Phi	0	Hydroxyl
Threonine	Thr	T	ACA	5.30	POU	Phi	p	Hydroxyl
Tryptophan	Trp	W	UGG	0.76	HPH	Pho	0	Aromatic
Tyrosine	Tyr	Y	UAC	3.79	POU	Phi	0	Aromatic
Valine	Val	V	GUA	3.79	HNP	Pho	0	Aliphatic

HNP-Hydrophobic nonpolar; POU-Polar uncharged; Pho-Hydrophobic; Phi-Hydrophilic.

Yamauchi *et al.* (1996), *Bombyx mandarina* (AC No. AF 001289) 623 Yamauchi *et al.* (1997), *Derobrachus geminatus* (AC No. L25278) 659 Smith *et al.* (1994), *Locusta migratoria* (JO 3888) 672 Kanost *et al.* (1988), *Galleria mellonella* (AJ 006975) 699 Niere *et al.* (1999), *Agrius convolvuli* (AF 001632) 537 Regland. *et al.* (1997), *Spodoptera litura* (AF 094582) 704 Kim *et al.* (1998), *Epiphyas postvittana* (AF 314181) 709 Liu *et al.* (2000), *Manduca sexta* (M17286) 708 Cole *et al.* (1987).



Gm	*Galleria mellonella*
Ac	*Argirus convolvuli*
Ad	*Acheta domesticus*
Lm	*Locusta migratoria*
Dc	***Dysdercus cingulatus <present study>***
Dg	*Derobrachus geminatus*
Sl	*Spodoptera litura*
Ep	*Epiphyas postvittana*
Ms	*Manduca sexta*
Bm	*Bombyx mori*
Bma	*Bombyx mandarina*

FIGURE 3. Phylogenetic tree of *Dysdercus cingulatus*.

Apolipophorin-III was isolated from haemolymph of *D. cingulatus* by KBr density gradient ultra- centrifugation and molecular weight was determined as 18 kDa with HPLC and SDS-PAGE (Mohan and Muraleedharan., 2001) This is also in full agreement with the molecular mass of apolipophorin III reported in other insect species.

Percentage similarity of nucleotide sequence of *D. cingulatus* apoLp-III gene was compared with the available ten sequences. Coding sequence of apoLp of *D. cingulatus* was closely related to *Derobrachus geminatus* and distantly related to

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B.mori      CATCATGGCCGCCAAGTTCGT---AGTTCTCTTCGCCTGCATCGCTCTGGCCCAAGGAGC 92
Bombyx      CATCATGGCCGCCAAGTTCGT---AGTTCTCTTCGCCTGCATCGCTCTGGCCCAAGGAGC 92
Agrius      CATCATGGCAGCCAAGTTCGTCGTGGTTCTCGCCGCGTGCGTGGCCCTCTCGCACAGCGC 5099
lipo16.D.c  ------------------------------------------------------------

B.mori      GATGGTGCGACGCGACGCTCCCG---------ACTTCTTCAAGGACATCGAACACCACAC 143
Bombyx      GATGGTGCGACGCGACGCTCCCG---------ACTTCTTCAAGGACATCGAACACCACAC 143
Agrius      GATGGTGCGCCGCGACGCTCCCGCTGGCGGCAACGCGTTTGAAGAGATGGAGAAGCACGC 5159
lipo16.D.c  ------------------------------------------------------CTA 3
                                                                     *
B.mori      CAAGGAGTTCCATAAGACTTTAGAACAACAGTTTAACTCGCTCACCAAGTCAAAGGACGC 203
Bombyx      CAAGGAGTTCCATAAGACTTTAGAACAACAGTTTAACTCGCTCACCAAGTCAAAGGACGC 203
Agrius      CAAAGAGTTCCAGAAGACCTTCAGCGAGCAATTTAACTCCCTCGTCAACTCCAAAAACAC 5219
lipo16.D.c  CAAATGTTTCTGCTAAACCAAGAGCCA--AAGTCGCCGAAAATCTCAACTGCAAGAACTT 61
            ***   ***   * **       *  *  *  *       *** *  **  **
B.mori      ----ACAGGACTTCAGCAAGGCTTGGAAG-GACGGCTCCGAGTCCGTGCTGCAACAGCTC 258
Bombyx      ----ACAGGACTTCAGCAAGGCTTGGAAG-GACGGCTCCGAGTCCGTGCTGCAACAGCTC 258
Agrius      ----CCAGGACTTCAACAAGGCGCTTAAG-GATGGCTCCGACTCCGTGCTGCAGCAACTG 5274
lipo16.D.c  GCCGCCAATGCCCAACCTAATTTTGAAAACAATAACAATAATTTTATATTAAAACAAT-- 119
              **   *   * * *       **   *   *   * *   *  *  * **
B.mori      AACGCCTTCGCCAAGAGTCTCCAGGGAGCGCTCGGAGACGCGAACGGCAAGGCCAAGGAG 318
Bombyx      AACGCCTTCGCCAAGAGTCTCCAGGGAGCGCTCGGAGACGCGAACGGCAAGGCCAAGGAG 318
Agrius      TCCGCCTTCTCCTCCAGTCTTCAGGGAGCGATAAGCGACGCTAACGGCAAAGCCAAGGAA 5334
lipo16.D.c  ---GTCGTTTATTCAATTTTATCAAGCAAACTGAGCGAGACCGCAGCTGAATTGCAAAAA 176
               * * *       * * *     *     *  * **  *    *   *     *  *
B.mori      GCTTTGGAACAGTCGAGGCAGAACATCGAGCGCACGGCCGAGGAGCTCCGCAAGGCCCAC 378
Bombyx      GCTTTGGAACAGTCGAGGCAGAACATCGAGCGCACGGCCGAGGAGCTCCGCAAGGCCCAC 378
Agrius      GCGCTGGAGCAGGCGCGCCAGAACGTAGAGAAGACCGCCGAGGAGCTGCGCAAGGCGCAC 5394
lipo16.D.c  CAGTTGGGAC---CTTGGCATAACATTCATTAAG-----GAAAAATCATGTGATTTTTAC 228
              ***  *   *  * ** *** *  *          **  *     *  *     **
B.mori      CCTGACGTCGAGAAGAACGCCACCGCCCTCCGCGAGAAGCTGCAGGCCGCCGTGCAGAAC 438
Bombyx      CCTGACGTCGAGAAGAACGCCACCGCCCTCCGCGAGAAGCTGCAGGCCGCCGTGCAGAAC 438
Agrius      CCCGACGTCGAGAAGGAAGCCAACGCGTTCAAGGACAAGCTGCAGGCGGCCGTGCAGACC 5454
lipo16.D.c  CTATAATCATAATTAACATCAAATGAGGGCCGTAAATATTTATTAACGATAATATATAGT 288
            *   *     *        * *  *    *    *  *  *     *      *  * *
B.mori      ACCGTGCAGGAATCCCAGAAGTTAGCGAAGAAGGTGTCCTCGAACGTGCAGGAGACTAAT 498
Bombyx      ACCGTGCAGGAATCCCAGAAGTTAGCGAAGAAGGTGTCCTCGAACGTGCAGGAGACTAAT 498
Agrius      ACCGTGCAGGAGTCCCAGAAGTTGGCGAAGGAGGTGGCTTCTAACATGGAGGAGACAAAC 5514
lipo16.D.c  AC---ATATAACTTTGAAGACCAAATTGTAGAAGTTAGTAACAACGTGCAACA-ACAAGT 344
            **     *  * *   *  *          * **       *** ** *  * ** *
B.mori      GAGAAACTGGCGCCCAAGATCAAGGCCGCCTACGACGACTTCGCGAAGAACACCCAGGAG 558
Bombyx      GAGAAACTGGCGCCCAAGATCAAGGCCGCCTACGACGACTTC-CGAAGAACACCCAGGAG 557
Agrius      AAGAAACTGGCGCCCAAGATCAAGCAGGCCTATGACGACTTCGTGAAGCACGCCGAGGAG 5574
lipo16.D.c  AAGAA----GAGCTATAGTTC---------------------------------------- 361
             ****    * **   ** **
B.mori      GTGATCAAGAAGATCCAGGAGGCCGCCAACGCCAAGCAGTGAGCGTCGATATTGAACTCT 618
Bombyx      GTGATCAAGAAGATCCAGGAGGCCGCCAACGCCAAGCAGTGAGCGTCGATATTGAACTCT 617
Agrius      GTGCAAAAGAAACTGCACGAGGCCGCCA---CCAAGCAGTGAGGAGGCTCCGCGCATGCG 5631
lipo16.D.c  ------------------------------------------------------------

B.mori      CACAC------------------------------------------------------- 623
Bombyx      CACAC------------------------------------------------------- 622
Agrius      CACACGAGTCCGCGCCGTTACCCGCGTTAACCTCACCCTGCACCACCGATACAATAAAGC 5691
lipo16.D.c  ------------------------------------------------------------
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FIGURE 4. Multiple sequence alignment of *D. cingulatus* apoLp-III with other insects. *B. Mori-Bombyx mori*, Bombyx–*Bombyx mandarina*, Agrius–*Agrius convoluvuli*, D.C–*D. cingulatus*

Epiphyas postvittana. Dendrogram comparison with other known species showed that both *D. cingulatus* and *Derobrachus geminatus* belong to related groups. All these data from the present study suggest that *D. cingulatus* and *Derobrachus geminatus* have evolved from a common ancestor. ApoLp-III gene of *D. cingulatus* shows the sequences producing significant alignment (bits value) with *Derobrachus geminatus*.

The amino acid composition of *D. cingulatus* lipophorin molecule showed considerable similarity to lipophorin from other insect species. *D. cingulatus* lipophorin has a higher proportion of aspargine (9.85%), lysine (8.33%), leucine (7.58%) as in agreement with that in *Blattella germanica, Manduca sexta, Locusta migratoria, Papilo polyxenes, Diatraea grandiosella* and *Gastrimargus africanus* (Chino and Kitazawa, 1981; Ryan *et al.*, 1986; Haunerland *et al.*, 1986; Shapiro *et al.*, 1988; Cole and Wells, 1990; Sevala *et al.*, 1999). The high percentage of isoleucine (15.15%) noticed in the present study again is in agreement with the data already known in *Diatraea grandiosella* (Shapiro *et al.*, 1988). *D. cingulatus* lipophorin showed low percentage composition of amino acids in histidine (1.51%), arginine (3.03%) and tyrosine (3.79%). Similar observations were reported earlier in various insect species (Cole and Wells, 1990; Sevala *et al.*, 1999).

ApoLp-III from all the insect species studied invariably lack cysteine. Our present finding also agrees with this observation. The percentage of methionine (1.51%), histidine (1.51%), tryptophan (0.76%) are also at very low levels. This again is in full agreement with previous observations (Beenakkers *et al.*, 1986; Haunerland *et al.*, 1986; Ryan, 1990).

ApoLp-III cDNA cloned gene shows sequence similarity and identity to insects from the related orders while showing divergence from certain orders. The identified nucleotide sequences and amino acid analysis in the present study showed the clone as a correct insect apolipophorin-III encoding gene. Dendrogram, phylogenetic tree and multiple sequence alignment all revealed the relationship with other known insect species and confirm the position of *D. cingulatus* in the evolutionary tree and the established taxonomic relations. It may be concluded that apoLp-III sequences has been well conserved in the same order, but diverged between the orders during evolution.

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Seasonal occurrence of two egg parasitoids, *Chrysochalcissa oviceps* Boucek and *Gryon* sp. of coreid bug (*Paradasynus rostratus* Distant) of coconut in Kerala

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ABSTRACT: A survey on seasonal occurrence of newly recorded egg parasitoids, *Chrysochalcissa oviceps* Boucek and *Gryon* sp. of coreid bug (*Paradasynus rostratus* Distant) of coconut was carried out at Instructional Farm, College of Agriculture, Vellayani, Trivandrum, Kerala. Parasitisation by *C. oviceps* peaked during April-May and extended till October. Parasitisation by *Gryon* sp. was observed from June to November with maximum parasitisation during October. Both parasitoids were found to be potential egg parasitoids of coreid bug. The diagnostic characters of the parasitoids are also discussed.

KEYWORDS: Egg parasitoids, *Chrysochalcissa oviceps*, *Gryon sp.*, Coreidae, *Paradasynus rostratus*

Coreid bug of coconut *Paradasynus rostratus* Distant is an important sucking pest causing high economic loss to coconut growers in Kerala. In India, *P. rostratus* was first reported as a pest of coconut from Alleppey district of Kerala (Kurien *et al.*, 1972, 1976, 1979). The nymphs and adults feed on young buttons through the perianth causing buttonfall. Infested buttons which remain on the bunches develop necrotic lesions with furrows, crinkles and gummosis (Kurien *et al.*, 1979). The attack of *P. rostratus* was estimated to cause 17.99 per cent loss in nut weight and 19.96 per cent loss in nut volume (Nair *et al.*, 1997), in addition to premature nutfall ranging from 13.5–62.5 per cent (CPCRI, 1999). Management measures include spraying endosulfan 0.1 per cent or carbaryl 0.05 per cent (Ponnamma *et al.*, 1985). However, economic and environmental concerns keep farmers away from adopting the recommended crown spraying. Detailed studies on biological control of coreid bug has not been carried out in India. Hence, attempts were made to identify the naturally occurring parasites of coconut coreid bug and explore the possibilities of their use as biocontrol agents.

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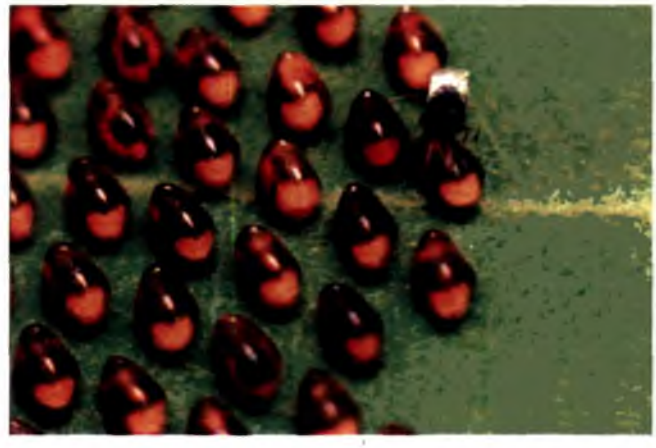

FIGURE 1. Parasitised egg mass of *Paradasynus rostratus* with female of *Gryon* sp. over it (Magnification 4x).

Periodic surveys were conducted to identify parasites of *P. rostratus* with special reference to potential egg parasitoids, in coconut and its other host plants like cashew, guava, tamarind, cocoa and neem. Egg masses of coreid bug were collected from the field and kept in polythene covers and observed for emergence of parasitoids. The parasitoids emerged from the eggs of coreid bug were collected and maintained in glass vials of 10 cm length and 2 cm diameter, streaked with honey–water (1 : 1) mixture. The adult females were released to fresh egg mass of coreid bug and observed for parasitisation.

The eggs of *P. rostratus* were parasitised by the parasitoids released to the fresh egg mass. The parasitised eggs turned brown in 4.66 days and completely black in 6.57 days (Fig. 1), while the unparasitised eggs turned reddish orange (Fig. 2). The adult parasitoids emerged from the eggs by cutting open an irregular hole on the egg surface, opposite to the operculum while the nymphs of coreid bug emerged by opening the black operculum of unparasitised eggs. Two different species of parasites identified were *Chrysochalcissa oviceps* Boucek (Fig. 3) and *Gryon* sp. (Fig. 4), belonging to Families Torymidae and Scelionidae respectively. Though the Scelionid was earlier recorded as *Gryon homeoceri* (Nixon) (Mohan *et al.*, 2001), examination of further material proved that the species is not *homeoceri* (T. C. Narendran, personal communication). Both *C. oviceps* and *Gryon* sp were solitary parasitoids. The diagnostic characters and features differentiating the two parasitoids are given in Table 1. After parasitisation *C. oviceps* took 18–24 days for emerging as adult, where as *Gryon* sp. took 20–25 days. In the laboratory, when fed with honey water mixture the adults of *C. oviceps* and *Gryon* sp. survived for 30–38 days and 30–34 days respectively.

Field exploration for a period of one year from April 2000 to March 2001 revealed seasonal variation in the extent of parasitisation (Table 2). Parasitisation by *C. oviceps*

TABLE 1. Diagnostic features of *Chrysochalcissa oviceps* Boucek and *Gryon* sp.

Chrysochalcissa oviceps

Length 1.4–1.8 mm. Head, thorax dark aeneous; setae on head, thorax, legs white; gaster, propodeum, all coxae, hind femur black with brownish tinge; mouthparts, antennae brownish, antennal scape lighter; all trochanters light brown; pro- and mesofemora dark brown with light brown distal end, hind femur black, all tibiae brown with lighter extremeties, all tarsi creamy white. Median ocellus placed outside scrobe; occipital margin excarinate behind eyes. Radicle of antenna thinner and shorter than pedicel, scape slightly shorter than scrobe, pedicel globose, annulus distinct; all funicular segments, except last, broader than long. An indistinct vertical carina extends from upper mouth margin to scrobe. Mesoscutum with notaular lines; mesoscutellum depressd near apex; wings hyaline, submarginal vein slightly longer than marginal vein, stigmal vein thin with expanded stigma forming a hook. First tergite of gaster glabrous, second with a transverse row of white setae near anterior margin, succeeding tergites with widely placed setae, gaster apparently shorter than mesosoma.

Gryon sp.

Length 1.54–1.70 mm. Head, mesosoma, metasoma entirely black; ocellus, pubescence white; antennae entirely yellowish brown in male, distal five flagellomeres black in female; mouthparts brown; wings hyaline, veins yellowish brown; legs yellowish brown excepting dark brown coxae, last tarsomere darker than preceding ones. Lateral ocellus separated from eye by a distance equal to diameter of one ocellus; median ocellus placed above scrobe seperated by a distance of two times diameter of one ocellus. Scrobe transversely striated with a vertical carina along middle; a weak vertical carina extends between median ocellus and scrobe. Radicle as long as pedicel, much thinner than pedicel, scape long, its distal end reaching upper margin of scrobe; pedicel longer than broad, slightly shorter than first flagellomere; second flagellomere apparently larger than wide; flagellomeres 3–7 in male apparently broader than long, 8–9 distinctly wider than long, last longer than wide. Mesoscutum, mesoscutellum longitudinally rugose; mesoscutellum with a row of large, deep punctures along posterior margin, slightly depressed near the apex; metanotum with a row of deep pits larger than punctures on posteror margin of mesoscutellum. Stigmal vein apically broadened.

Sexual dimorphism: female slightly larger than male, disal five flagellomeres black in female (antenna entirely yellowish in male); flagellomeres 2–9 distinctly wider than long in female, 3rd and 4th separately being two times as broad as long (flagellomeres 3–7 separately only slightly wider than long in male).

Chrysochalcissa can be differentiated from *Gryon* by the following characters

	Chrysochalcissa oviceps	*Gryon* sp.
1.	Anntenal sockets situated in the middle of eyes	Antennal sockets placed slightly below the lower level of eyes
2.	Scape shorter than transverse diameter of eye	Scape distintly longer than transverse diameter of eye
3.	Scape shorter than half of flagellum	Scape distintly larger than half of flagellum
4.	Notauli present	Notauli absent
5.	Posterior margin of pronotum completely visible in dorsal view.	Pronotum extremely narrow, middle of its posterior margin concealed by mesonotum in dorsal view
6.	Gaster not dorsoventrally flattened	Gaster distinctly dorsoventrally flattened
7.	First and second tergite of gaster without longitudinal lines.	First and second tergite of gaster with characteristic longitudinal lines
8.	Hind femur highly enlarged, toothed	Hind femur not greatly enlarged, not toothed
9.	Hind tibia curved.	Hind tibia straight
10.	Gaster not margined ventrally.	Gaster distinctly margined ventrally

FIGURE 2. Egg mass of *Paradasynus rostratus*.

FIGURE 3. Adult of *Chrysochalcissa oviceps* (Magnification 45x).

peaked during April–May and extended till October. Parasitisation by *Gryon* sp. was observed from June to November with maximum parasitisation during October. The extent of parasitisation of eggs in an egg mass ranged from 84.8–100 per cent and 50–100 per cent for *C. oviceps* and *Gryon* sp. respectively. Visalakshi *et al.* (1989) reported that the population of coreid bug reached a maximum during the months of August, September and October and declined from the month of November onwards reaching low levels during January to May as influenced by the weather parameters such as rainfall, temperature and relative humidity. In addition to the influence of weather parameters, the extent of parasitisation may also have a role in regulating

FIGURE 4. Adult female of *Gryon* sp. (Magnification 75x).

TABLE 2. Natural parastisation by *Chrysochalcissa oviceps* and *Gryon sp.* in the egg mass of *P. rostratus*

Months*	Per cent egg mass parasitised by		Per cent eggs parasitised in egg mass by	
	C. oviceps	*Gryon* sp.	*C. oviceps*	*Gryon* sp.
April	50	0	97.28	—
May	66.66	0	100	—
June	20	20	95	100
July	25	20	84.8	98.1
August	40	0	100	—
September	0	20	—	50
October	25	50	88.88	100
November	0	20	—	98

*Egg masses could not be detected in the field during the months of January, February, March and December.

field population of coreid bug. This is indicated by comparatively low nut fall due to coreid bug infestation during peak periods of parasitisation (unpublished data).

Nair and Remamony (1964) recorded two species of egg parasitoids *viz. Hadrophanurus* sp and *Anastatus* sp. as natural enemies of *P. rostratus.* Possibilities of the use of egg parasitoid *Oenocyrtus albicrus* (Prinsloo) (Hymenoptera, Encyrtidae) for the management of *Pseudotheraptus wayi* Brown, another coreid bug of coconut in Zanzibar was discussed by Oswald (1990). Neethling and Joubert (1994) reported two egg parasitoids, *Anastatus* sp. and a scelionid causing natural mortality of *P. wayi.* Studies are currently undertaken for exploring the potential *C. oviceps* and *Gryon sp.* as a component of BIPM in coconut.

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Susceptibility of larvae and pupae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* to leaf extract of *Ocimum canum* Sims.

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ABSTRACT: The susceptibility of various larval stages and pupal stages of three species of mosquitoes, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* was determined by treatment with varying doses of leaf extract of an indigenous plant, *Ocimum canum* Sims. belonging to family Lamiacae. The second instar of *A. aegypti* was found most susceptible followed by *A. stephensi* and *Culex quinquefasciatus*.

KEYWORDS: Larvicidal activity, *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus*, *Ocimum canum*

Various plants have been screened and explored for their insecticidal property. Plant products being non-hazardous to non-target animals can be used more safely. They enjoy bio-suitability and hence are eco-friendly in nature. The present investigation was done with the objective of identifying more plants for insect population control. The extract was administered on larval and pupal stages because the best option for mosquito control is to target these stages, rather than the adults.

The second, third and fourth instar larvae and pupae (0–18 h) were treated with acetone leaf extract of *Ocimum canum* Sims. obtained by Soxhlet's extraction method. The various stages of larvae were reared in enamelled trays and yeast was provided as food. A batch of 20 larvae of each stage and similar number of pupae (for replicates for each treatment) were treated with various doses of the extract of *Ocimum canum* prepared from the stock solution. Each treatment including control were replicated four times. A constant environment was maintained during the rearing and experiment by keeping the temperature and humidity at 38 ± 2°C and 80 ± 5%, respectively. Light was provided by two 40 W fluroscent tubes and a 60 W bulb. The light and dark periods were maintained in the ratio of 10 : 14 hrs per day. Mortality counts were made 24 h after the treatment.

The larval and pupal stages of all the three species of mosquitoes were found highly susceptible to the leaf extract of *Ocimum canum* (Table 1) From the data presented

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TABLE 1. Mortality of mosquito larvae in different concentrations of *Ocimum* extract

Treatment	Mean of mortality			
	Anopheles stephensi			
Ocimum extract (ppm)	II Instar	III Instar	IV Instar	Pupa
600	—	—	86.25	76.25
500	—	—	72.50	60.00
400	—	71.25	48.75	42.5
300	71.25	53.75	36.25	27.5
200	50.00	35.00	23.25	—
100	17.50	11.25	—	—
CD at 5%	5.488	5.737	3.278	3.102
	Aedes aegypti			
600	—	—	88.75	95.00
500	—	—	73.75	76.25
400	—	81.25	58.75	46.25
300	77.50	51.75	37.50	31.25
200	53.75	38.75	25.00	—
100	18.75	8.75	—	—
CD at 5%	4.456	6.824	5.086	6.319
	Culex quinquefasciatus			
600	—	—	77.5	68.75
500	—	—	65.00	51.25
400	—	68.75	41.25	38.75
300	63.75	50.00	35.00	26.25
200	48.75	32.50	23.75	—
100	17.50	8.75	—	—
CD at 5%	5.348	4.034	4.436	4.753

Date are based on four replicates of 20 insects, each.

in the table, it is clear that the second instar larvae of all three species are the most susceptible. Susceptibility reduces in advanced stages of development like third instar, fourth instar and pupae. The larvicidal and pupicidal action of *Ocimum* extract marks it to be a promising larvicide and pupicide.

Earlier, the larvicidal activity of garlic against *Aedes aegypti* has been reported by Amonkar and Reeves (1970). Neem and by products have been found very active larvicide by Attri and Ravi Prasad (1980). Ayyangar and Rao (1989), Saxena *et al.* (1991). Various plant extracts were also found to suppress mosquito population (Saxena and Sumithra, 1985a,b; Saxena and Yadav, 1983). *Ocimum* extract was tried for the first time in this study.

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Kiwifruit, *Actinidia deliciosa* Chev. as a host of California red scale, *Aonidiella aurantii* (Maskell)–A new record

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ABSTRACT: California red scale *Aonidiella aurantii* (Maskell) infestation was recorded on the kiwi fruit vine *Actinidia deliciosa* Chev. from Solan (Himachal Pradesh), situated at an elevation of about 1400 m amsl, in the northern part of the India. The population of the scale was sparse (3–4/cm^2) on the twigs. This is a first record of the pest on kiwifruit vine from the world.

KEYWORDS: Kiwifruit, *Actinidia deliciosa*, *Aonidiella aurantii*, California red scale

Kiwi fruit, *Actinidia deliciosa* Chev., also known as Chinese gooseberry is a native of Central China and commercialized for the first time in New Zealand. It is also designated as health fruit due to high vitamin C content and other medicinal values. In Himachal Pradesh, India, its cultivation was undertaken by Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni (Solan) somewhere in 1985 and is now recommended for cultivation mainly in the mid-hill regions (1000–1500 m amsl) of the state. Kiwifruit wine is infested with a few insect pests at different stages of its development but none of them is a major pest.

In November 2003, an armoured scale commonly known as California red scale, *Aonidiella aurantii* (Maskell) (Diaspididae: Hemiptera), a well established pest of citrus group, was recorded for the first time infesting kiwifruit twigs at Solan (1400 m amsl). Both females (oval armour with central nipple) as well as males (slightly elongate armour with eccentric nipple) were observed on the twigs, which were covered with orange test (scale cover). On lifting the scale cover, the yellow insect was visible below it and underneath some females, the yellow crawlers were also observed under the microscope. Around the feeding point, the ashy grey spot was observed on the twigs.

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The scale population was sparsely distributed (3–4 scales/cm^2) on the twigs and is likely to increase with the passage of time as many females were in the reproductive phase. It indicated the suitability of kiwifruit vine for development of *A. aurantii*.

It is a first record of the scale, *A. aurantii* infestation on kiwifruit vine from the world though other diaspidids namely, greedy scale, *Hemiberlesia rapax* Comstock, latania red scale *H. lataniae* Signoret and oleander scale, *Aspidiotus nerii* Bouche have been reported from New Zealand (Tomkins *et al.*, 1997), Blank *et al.*, 1999; Steven, 1999). Another scale, *Pseudaulacaspis pentagona* (Targioni-Tozzetti) has been reported on kiwi vine from northern Greece, infesting shoots, fruits and leaves (Paloukis *et al.*, 1997).

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New records of butterflies in the Andaman and Nicobar Islands Bay of Bengal, Indian Ocean

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ABSTRACT: About 215 species of butterflies are known from the Andaman and Nicobar islands. The Andaman butterfly fauna is better known than that of the Nicobars. *Junonia lemonias* is being recorded for the first time from these islands while three species of butterflies (*Phalanta phalantha phalantha, Paduca fasciata* and *Euploea phaenareta phoebus*) hitherto thought to be vagrants are now being confirmed as residents on these islands thus adding 4 new species to the butterfly fauna of these islands. The larval food plants of all these species have been discovered. We report the presence of 16 species of butterflies on islands on which they were so far not known to occur. © 2004 Association for Advancement of Entomology

KEYWORDS: Butterflies, new records, range extensions, Andamans, Nicobars

The Andaman and Nicobar islands have been identified as one of the six megadiversity hotspots of India and are listed among the eight hottest hotspots in the world (Myers *et al.*, 2000). A total of about 215 species of butterflies are known from these islands (cf. references in Prashanth Mohanraj and Veenakumari (1996)). The only authentic additions to the butterfly fauna after the British worked these islands are *Delias hyparete* (Linnaeus 1758), (Singh and Khatri 1987) and *Erionota thrax thrax* (Linnaeus 1767), (Veenakumari and PrashanthMohanraj 1991). In addition species like *Pachliopta coon sambilanga* Doherty 1886, *Polyura schreiber tisamenus* (Fruhstrofer 1914) and *Lethe europa tamuna* de Niceville 1887 which were though to be stragglers or had not been seen for several years after their first sightings were recently confirmed as residents on these islands (Veenakumari and Prashanth Mohanraj, 1994, 1996, 1997).

We now report on the occurrence of 17 species of butterflies on islands on which they were not known to occur before (Table 1). Three of these species, *Phalanta phalantha phalantha* (Drury 1773), *Paduca fasciata* (C. Felder and R. Felder 1860) and *Euploea phaenareta phoebus* Butler 1866, were thought to be vagrants on these

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islands. We have for the first time found breeding populations of these three species on the islands on which they are being recorded. The larval food plants of all the three species are being recorded for the first time from these islands. Not only did we find populations of the immature stages of these species on their larval food plants in the field but also successfully reared them through to adulthood in capacity on the plants on which they were found. The larval food plants of four more species have also been found on the islands on which they are now being newly recorded. These food plants, however, are not different from those on which they have previously been recorded by us (Veenakumari and Prashanth Mohanraj, 1997). With the exception of *Catopsilia pomona* (August) and *Paduca fasciata* (January), the larvae of all the other species were noticed in December.

Junonia lemonias Linnaeus 1758 which appears to be confined to Grant Nicobar is being recorded by us for the first time from anywhere on these islands. This is the fourth species of *Junonia* that is definitely known to be resident on these islands and the third species of this genus known to occur on the Nicobars. *J. orithya ocyale* Hubner is a fifth species that has so far only been taken as a straggler on Car Nicobar (Ferrar, 1948). The larval food plant of *J. lemonias* on these islands was tentatively identified by us as *Nelsonia campestris* R. Br. (Acanthaceae). We could not confirm the identity of this plant.

Seven species of *Arhopala* have been reported from the Andaman islands, of which *A. zeta* (Moore 1877) is an endemic. For the first time we report on the present of at least one species of *Arhopala* on the Nicobar islands. The identity of this species, which occurs in Great Nicobar, could not, however, be confirmed. *Danaus melanippus* (Cramer 1777) is represented by the endemic subpecies *D. melanippus camorta* (Evans, 1932) in the Central Nicobars (Vane-Wright, 1993). There is only one say record of this species from the Andaman islands with a record from North Andaman (Khatri and Mitra, 1989). We have found resident populations of this species on South Andaman and have successfully reared it in capacity on *Sarcolobus carinatus* (Griff.). Whether the Andaman population represents an undescribed subspecies as surmised by Vane-Wright (1993) is yet to be determined.

Our findings reinforce the fact that the Nicobars have not been studied as intensively as the Andamans and that when studied adequately, will yield many more species. Fifteen of the seventeen species that we report here are being recorded on islands in the Nicobars. Only two species, *P. phalantha* and *D. melanippus* are confirmed additional records for the Andamans. With these findings we raise the total number of butterfly species on the Nicobars from 99 to 108 and on the Andamans from 180 to 182. We urge that the Nicobar islands be surveyed for their butterfly fauna as also the outlying islands of the Andaman group which continue to remain 'partially or completely unworked' after Ferrar (1948) departure from these islands in 1931.

The supreme Court of India has in a recent land mark judgement ruled against the felling of trees in the Andaman and Nicobar Islands (Anonymous, 2002). This augurs well for the unique flora and fauna of these islands. Nevertheless, plans are afoot to develop these islands for tourism as a result of which many areas, some pristine or

TABLE 1. New records and range extensions of butterflies in the Andaman and Nicobar islands

Species	Previous distribution	New record
Hesperiidae		
Coeliadinae		
Badamia exclamations (Fabricius, 1775)	S. Andaman	Great Nicobar
Hesperiinae		
Notocrypta curvifascia (C. and R. Felder, 1862)	Andamans	Great Nicobar (Breeding population) Lfp. *Zingiber* sp. Zingiberaceae
Erionota thrax (Linnaeus, 1767)	Andamans	Great Nicobar
Lycaenidae		
Polyommatinae		
Neopithecops zalmora (Butler, 1870)	Andamans	Car Nicobar, Great Nicobar
Theclinae		
Arhopala sp.	Andamans (all 7 species)	Great Nicobar
Pieridae		
Pierinae		
Leptosia nina nicobarica Doherty, 1886	S. Nicobars	Kamorta (Central Nicobars)
Coliadinae		
Catopsilia pyranthe (Linnaeus, 1758)	S. Andaman	Katchal (Central Nicobars)
Catopsilia pomona (Fabricius, 1775)	Andamans	Great Nicobar
Nymphalida		
Nymphalinae		
Doleschallia bisaltide andamanensis Fruhstorfer, 1899	Andamans, Car and Central Nicobars	Great Nicobar (Breeding population) Lfp. *Pseuderanthemum album* (Nees Merr., Acanthaceae)

TABLE 1. Contd...

Species	Previous distribution	New record
Junonia atlites (Linaeus, 1763)	Andamans, Nankauri (Central Nicobars)	Great Nicobar
Junonia atlites Linnaeus, 1758		Great Nicobar (Breeding population)
Phalanta phalantha phalantha (Drury, 1773)	Central Nicobars (Straggler)	South Andaman (Breeding population) Lfp. *Flacourtia ramontchi* L., Flacourtiaceae
Cirrochroa nicobarica Wood-Mason and de Nicelille, 1881	Great Nicobar (S. Nicobars) Katchal (Central Nicobars)	
Paduca fasciata (C. Felder and R. Felder, 1860	S. Andaman (Straggler)	Kamorta (Central Nicobars) (Breeding population) Lfp. *Ryparosa javanica* (B.I.) Kurz ex Koord. & Van. Flacourtiaceae
Danainae		
Euploea phaenareta phoebus Butler, 1866	Central Nicobars (Katchal and Nanauri) (Straggler)	Great Nicobar (Breeding population) Lfp. *Cerbera manghas* L., Apocynaceae
Danaus chrysippus chrysippus (Linnaeus, 1758)	Great Andaman, Car and Central Nicobars	Great Nicobar (Breeding population) Lfp. *Calotropis gigantea* (Wild.) Dryand ex Ait., Asclepiadaceae
Danaus (Salatura) melanippus (Cramer, 1777)	N. Andaman; Central Nicobar (Nankauri), Great Nicobar	South Andaman (Breeding population) Lfp. *Sarcolobus carinatus* Griff., Asclepiadaceae

*Not recorded previously from anywhere in the Andaman and Nicobar islands Lfp. larval food plant

near pristine, which were earlier prohibited to tourists would be thrown open. Since the Andaman and Nicobar Islands are small islands their fauna will not be able to withstand the pressures of increased tourist traffic. The fact that even butterflies, which along with snails are the best known invertebrate groups (McVean, 1976) on these islands, are still not adequately known, indicates that unless effective conservation measures are taken, many species of both plants and animals may disappear even before they are discovered.

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Laboratory screening of newer insecticides against the red palm weevil, *Rhynchophorus ferrugineus* Oliv.

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ABSTRACT: Commercial grade formulations of acephate, triazophos, chlorpyriphos, carbosulfan and imidacloprid @ 0.05 and 0.01% were evaluated against the adults and third and fifth instar grubs of red palm weevil *R. ferrugineus*. The results showed that the mortality in carbosulfan 0.05% was significantly superior in the case of adult weevils and fifth instar grubs. Cent percent mortality was observed in the case of adult weevil. In the case of grubs, carbosulfan 0.05% resulted in 96.67 and cent per cent mortality of the fifth instar when treated by direct spray and feed dip method, respectively. The mortality of third instar grubs recorded on carbosulfan 0.05% was at par with imidacloprid 0.05% and acephate 0.05% in both the methods of testing. The mortality recorded in the lower dose also indicated the superiority of carbosulphan 0.01%.

KEYWORDS: Red palm weevil, *Rhynchophorus ferrugineus* carbosulfan, acephate, imidacloprid, chlorpyriphos, triazophos

Coconut is subject to attack by an array of pests of which the red palm weevil, *Rhynchophorus ferrugineus* Oliv. is considered one of the most destructive. (Nirula, 1956; Kurian, 1961). The management strategies against red palm weevil include both preventive and curative measures. The earliest method for its control in coconut was stem injection of pyrethrin, piperonyl butoxide combinations (Pyrocon E) at 1000–1500 ml per palm (Nirula, 1956). Leaf axil filling with a mixture of BHC 5% dust and sand was recommended as a prophylactic measure (Mathen and Kurian, 1959). Since then, a series of recommendations have been made as prophylactic or curative measures. Thus carbaryl 1% stem injection (Mathen and Kurian, 1967), trichlorphon 0.2%, carbaryl 0.1% or endosulfan 0.1% stem injection (Sathiamma *et al.*, 1982), Supracide, salut, metasystox and trichlorphon (Vidyasagar, 1998) were recommended. Since most of the above insecticides are either banned, under restricted use or not

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available in the market, the present study was conducted to evaluate some of the promising new insecticides against *R. ferrugineus.*

Commercial formulations of five insecticides, *viz.*, acephate, triazophos, chlorpyriphos, carbosulfan, and imidacloprid were evaluated against the grubs and adults of red palm weevil.

The initial culture of red palm weevil *R. ferrugineus* was raised by collecting adult weevils from infested coconut palms. They were maintained in the laboratory on cut pieces of leaf petiole of coconut. Third and fifth instar grubs and adults were used for the study.

Commercial grade formulations of acephate (Acetaf 75 SP, Rallis India Ltd.,), triazophos (Hostathion 40 EC, Aventis Crops Science India Ltd), chlorpyriphos (Tafaban 20 EC, Rallis India Ltd.), carbosulfan (Marshal 25EC, Rallis India Ltd.), and imidacloprid (Confidor 200SL, Bayer India Ltd.) were used. Two concentrations, 0.05 and 0.01% of each chemical was prepared by mixing the required quantity of the chemical with water. Direct spraying and feed dipping method were followed for treating the grubs. Dry film technique was followed in the case of adult weevils.

In the direct spraying method, the insecticide solutions were sprayed directly on ten numbers of third instar or fifth instar grubs taken in a clean petridish, using an atomizer. Three replications were maintained in each case. Grubs sprayed with water served as control. The treated grubs were kept exposed under a fan for the spray fluid to evaporate and the grubs were then transferred individually to small vials to avoid cannibalism and small coconut petioles were provided as food. In the feed dipping method, fresh coconut petioles of 15 cm length and 5cm breadth were dipped in the respective insecticide solutions for 24 hours and air dried for 10 min. The grubs were introduced into the treated petiole, one in each by piercing the treated petiole at one end using a pointed knife. Ten numbers of each instar represented one replication and there were three replications in each case.

In the dry film technique, three ml of the respective insecticide solution prepared by dissolving the chemical in acetone was transferred to glass vials, 15 cm × 8 cm. The containers were rotated horizontally so that a dry film of the toxicant was formed uniformly inside the vial. Ten numbers of healthy one day old adult weevils were released to the vial and fresh coconut petioles were given as food. Each treatment was replicated thrice. All the experiments were done in Completely Randomized Design. Mortality was recorded 48 hours after treatment.

The corrected mortality in all the experiments was calculated using Abbot's formula (Abbot, 1925). The data were subjected to statistical analysis using ANOVA.

The data presented in Table 1 showed that the mortality in carbosulfan 0.05% was significantly superior to other insecticides in the case of adult weevils and fifth instar grubs. Carbosulfan 0.05% resulted in cent percent mortality of adult weevils. In the case of grubs, it resulted in 96.67 and cent per cent mortality of the fifth instar when treated by direct spray and feed dip method, respectively. The mortality of third instar grubs recorded in the treatment was at par with imidacloprid 0.05% and acephate 0.05% in both the methods of testing. A more or less similar trend was observed when

TABLE 1. Mean mortality of adults and grubs of red palm weevil treated with insecticides

Insecticide and Dose	Mortality at 48 HAT				
	Adult	Third instar		Fifth instar	
	Dry film	Direct spray	Feed dip	Direct spray	Feed dip
Acephate 0.05%	8.02(63.25)	9.54(90.11)	9.73(93.67)	9.18(83.27)	8.22(66.57)
Chorpyriphos 0.05%	6.37(39.59)	9.59(90.91)	8.98(79.64)	6.65(43.22)	5.52(29.47)
Triazophos 0.05%	6.13(36.51)	8.05(63.80)	9.56(90.39)	8.42(69.69)	8.43(10.76)
Carbosulfan0.05%	10.05(100)	8.66(70.00)	9.89(96.81)	9.88(96.67)	10.05(100)
Imidacloprid 0.05%	7.59(56.56)	9.56(90.39)	9.35(80.42)	7.36(53.17)	7.81(59.99)
CD(0.05)	0.8805	0.4347	0.8504	0.652	0.759
Acephate 0.01%	8.22(66.57)	4.82(22.23)	4.08(15.65)	2.54(5.45)	1.77(2.13)
Chorpyriphos 0.01%	6.87 (46.80)	3.74(12.99)	6.73(44.29)	2.54(5.45)	1.77(2.13)
Triazophos 0.01%	7.12(49.69)	5.85(33.22)	4.93(23.30)	3.74(12.99)	2.54(5.45)
Carbosulfan 0.01%	10.05(100)	9.53(89.82)	8.84(77.14))	8.81(76.62)	8.01(63.10)
Imidacloprid 0.01%	8.02(63.32)	6.13(36.57)	4.93(23.30)	4.49(19.16)	4.16(16.31)
CD(0.05)	1.0243	1.0907	4.4504	2.1385	2.0218

Figures given are transformed ($\sqrt{x+1}$) values, with percentages within parentheses.

the insecticides were tested at their lower dose. The mortality recorded in the lower doses also indicated the superiority of cabosulfan 0.01%. Cent per cent mortality of adult weevil was observed in carbosulfan 0.01%. Significantly higher mortality of 76.62 and 63.10% of fifth instar were observed by direct spraying and feed dip method, respectively. In the case of third instar grubs, mortality recorded was significantly superior when treated by direct spraying while it was on par with imidacloprid 0.01%, acephate 0.01% and chlorpyriphos 0.01% when treated by feed dip method. The present study indicated the suitability of carbosulfan in the management of red palm weevil. Carbosulfan is a proinsecticide to carbofuran and is rapidly absorbed by the plant and slowly get converted to carbofuran. The effectiveness of carbofuran for use in pheromone traps was reported by Oehlschlager *et al.* (1993); Abraham and Nair (2001); Faleiro and Satarkar (2002).

Compared to carbofuran, it is less toxic and hence safe for application and has increased residual time also. Its application by way of stem injection, leaf axil filling or in pheromone traps can be advisable as it was found as the best. However, its effectiveness may be evaluated in the field.

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Contents of Volume 29

No. 1

SHORT COMMUNICATIONS

No. 2

No. 3

No. 4

AUTHOR INDEX

Reg. No. 123SBN

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